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THERAPY PHYSICS BIOLOGY

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CLINICAL TRIALS IN RADIOTHERAPY AND THE MERITS OF HIGH ENERGY PROTONS

by

S. GRAFFMAN and B. JUNG

The technical developments in radiotherapy have largely been aimed at allowing an adequate dose to be administered *optimally* (homogeneously or graded) to a volume suspected of tumour growth while avoiding excessive dose to surrounding tissue. With the ^{60}Co -sources, linear accelerators and betatrons now available at most radiotherapeutic centres, this object is achieved almost to the extent possible with photons and electrons. If further advancement along this line of progress is of importance, the logical next step would be to exploit ions such as protons accelerated to high energies. With this type of radiation the shaping of the radiation field can be made even more sophisticated.

High energy protons do not differ appreciably from other low LET radiations with respect to the biological effect and hence do not share the potential capacity of neutrons, negative pions and heavy nuclei with respect to the control of anoxic tumours. On the other hand, the biological effects can probably be modified by temporal and chemical means in the same manner as the effects of other low LET radiations.

During the last ten years, 180 MeV protons have been used for radiotherapy on a moderate scale at the Gustaf Werner institute. The experience is that the

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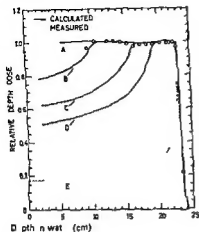


Fig 1 Depth dose curves for 185 MeV protons unmodified (E) and modified by the use of ridge filters (A—D) (From KARLSSON 1964 Courtesy Strahlen therapie)

thickness in the beam the peak can be swept in the axial direction over a suitable depth. The water absorber can be replaced by ridge filters which introduce different thicknesses of absorbing material in different parts of the beam (LARSEN 1961 KARLSSON 1964). Homogeneous depth dose distributions are obtained by a transversal oscillation of the filter. A typical dose distribution of regular shape measured in a water phantom is given in Fig 2.

The LET distribution of high energy protons (> 15 MeV) is nominally of the low LET type (< 3.5 eV/nm) (ICRP ICRU 1963). Recoil protons as well as nucleons and nuclei emitted in nuclear reactions add a small high LET com-

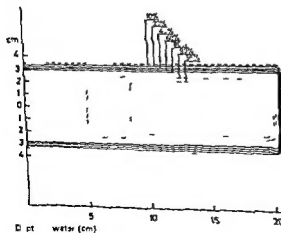


Fig 2 Depth-dose distribution for 185 MeV protons in water after a heavy alloy filter giving a 23 cm plateau

achieved dose distributions are favourable as judged by conventional standards, that the clinical results are comparable to those obtained with other types of radiation, and that no extra risks are involved, neither with respect to untoward effects nor to the safety of patient and personnel (FALKNER et coll 1962, FORS et coll 1964, GRAFFMAN et coll 1965). Contradicting evidence has not been reported from the other places where therapeutic irradiations by high energy protons have been performed (KJELLBERG et coll 1962, 1965, 1966, 1967, TOBIAS et coll 1964, 1967).

No strict quantitative study of how high energy protons compare with other types of radiation with respect to clinical results has been made. The irradiation facilities and the patient material have not allowed clinical trials (PATERSON 1958) to be set up and no other scientific method appears to be available.

It has been our aim to evaluate under what circumstances a clinical trial would be practical. To this end the relevant knowledge of the physical, biological and clinical properties of high energy protons was summarized and a number of tentative dose distributions constructed. With this approach, no quantitative estimate could be given, however, of the difference in clinical result that can be expected from the use of high energy protons as contrasted to other high energy radiations of more common use. The problem was therefore attacked by a rather general tumour therapy model and also by re-assessment of data from published clinical trials.

Physical properties of the high energy proton radiation field. The mono-energetic proton beam has a depth dose distribution of the Bragg type (cf Fig 1). The lateral scattering is small and in a first approximation the protons can be considered as moving in straight lines for the full penetration depth. The range of secondary particles, except those created in the rather few nuclear reactions, is less than 0.6 mm in soft tissue. The contribution to the dose from nuclear reactions is comparatively small both as regards secondary radiation ($\approx 20\%$) and remaining radioactivity ($\approx 0.2\%$) (HALLER & JUNG 1963, JUNG 1963, GRAFFMAN & JUNG 1967). The cross section of the field is easily defined by collimators, 50 mm of brass stopping a 185 MeV beam completely. The maximum penetration depth in tissue of such a beam is 23 cm.

The proton fluence over the utilized cross section can be made homogeneous by scatterers or by sweeping a pencil shaped proton beam in a reticular pattern with two variable, crossed magnet fields. In the latter case a transversal homo-

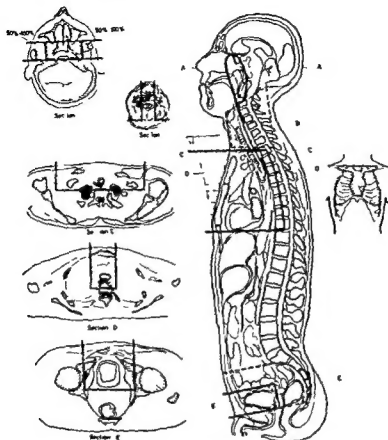


Fig 4 Schematic dose distributions for the treatment of nasopharyngeal oesophageal and bladder tumours

The dose to the lateral parts of the head can be $< 50\%$ of the dose to the nasopharynx if two opposing and overlapping fields with e.g. a 5 cm plateau are used

The broken line in the neck region indicates the field for irradiating the lateral lymph nodes. This can be achieved either by a split frontal field or by two continuous opposing lateral fields covering both the nasopharynx and the neck nodes

The hatched area in front of the thorax symbolizes a mould

The broken line in the pelvic region indicates the extension of the proton field for irradiation of large pelvic tumours

The heavy or broken lines indicate the extension of the homogeneous radiation field carrying the full tumour dose unless otherwise is specified

such as ^{60}Co photons. It can also be noted that superficial tissue seems to well tolerate the constant-dose proton field when in the therapeutic dose interval

More conclusive evidence has been obtained in animal and plant experiments. It has been demonstrated that the RBE value for high energy protons when compared to ^{60}Co photons, is close to 1.0 for most of the systems studied (cf

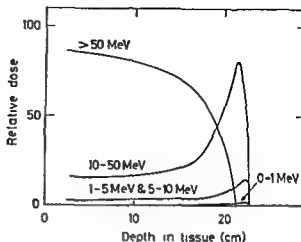


Fig. 3. Depth dose curves for various LET components for 185 MeV protons in soft tissue after a heavy alloy filter giving a 23 cm plateau. The calculations are based on ZERBY & KINNEY (1965). The curves for 5 to 10 MeV and 1 to 5 MeV protons are identical.

ponent. In the last few millimetres of their range the protons have themselves a high LET. The ridge filters and the variable water absorber modify the proton spectrum so that protons stop everywhere in the plateau region. More protons stop in the deeper layers and the high LET proton component hence increases with depth. The dose fractions due to protons of various energies is given in Fig. 3 as a function of depth in a flat dose distribution. The curves are based on data given by ZERBY & KINNEY (1965).

Knowing the appropriate RBE value in the different parts of the Bragg curve it would be possible to correct the shape of the filters so that the resulting depth dose distribution gives a constant biological effect in the plateau region. The increase in RBE is probably of importance only in the last few millimetres of the field in which the dose is already decreasing (GRAFFMAN *et al.* 1967).

In conclusion, high energy protons offer an outstanding flexibility in the shaping of the dose distribution. The beam cross section as well as the surface of maximum penetration of the field can easily be formed to an intricate shape by appropriate diaphragms and range determining moulds. The depth distribution can be varied continuously from one of the Bragg type to one of constant dose.

Radiobiological information on the effect of high energy protons. The use of high energy protons in radiotherapy has been limited and hence the information is meagre regarding their radiobiological effects in therapy, a fact which is further accentuated by the difficulties met with in the interpretation of the results. From the experience in Uppsala, it may be stated, however, that no clinical effects have been encountered which would indicate a marked dissimilarity in radiobiological effect between high energy protons and other low LET radiations.

etration depths at the different parts of the two opposing fields. Also tumours within other parts of the pharynx can of course, be irradiated according to similar principles.

Oesophagus and pulmones Since more than 40 % of the tumours in the oesophagus have an early lymphatic spread to mediastinal nodes (MERENDINO et coll 1952, TOMLINSON & WILSON 1945) there are often good reasons to include the whole mediastinum in the primary radiation field. This can be achieved with a single field as indicated in Fig 4. The dose to the spinal medulla is negligible and only a very small part of the lung is irradiated. The integral dose is reduced to about 1/10 of the integral dose in conventional therapy (PIERQUIN et coll 1966, LEDERMAN et coll 1966) although a larger volume is given full tumour dose. However a considerable part of the heart is included in the frontal proton field and an opposing back field may be advisable. The risks of heart injury have been discussed by e.g. MICHAÏLOV (1963) and STEWART et coll (1967) and according to their data the back field should carry about 30 % of the tumour dose. The dose to the spinal medulla given by this back field is well within the tolerance limits. In the treatment of bronchial carcinoma a similar but adequately extended field arrangement could be used.

Vesica urinaria and uterus Bladder tumours can be treated with a single field of protons without considerable irradiation of the rectal mucosa. When there is extended growth with regional lymphatic spread the whole pelvis can be irradiated with a broad single field. This technique has been applied in the treatment of cervical carcinoma (FALMER et coll 1962 and FORS et coll 1964). In this case extra bolus might be added to lower the dose to at least part of the rectal region.

Quantitative aspects on beneficial effects from proton radiotherapy

The four criteria for field selection seem to be generally accepted but there is a remarkable lack of quantitative reports of their importance in the literature.

Disregarding with reference to an earlier section any radiobiological differences between high energy protons, electrons and gammas, the major merit of the proton radiation field lies in its geometrical properties. These often allow the primary tumour to be given a homogeneous dose with very little dose to most of the surrounding tissue and the regional metastases to be better covered by the radiation field. There appears to be no quantitative report in the literature on the importance of these points. Of course some kind of cell survival model could be applied but as has been shown by e.g. SURT et coll (1967), the result of such model calculations often does not agree with the clinical experience. A

e.g. BONFT MAURY *et coll* 1964, JOHANSSON & LARSSON 1968, LARSSON 1967, STENSON 1969). It thus appears both reasonable and safe to adopt an RBE value of 1.0 ± 0.2 for use in tumour therapy although there are some reports of substantial differences from this value (BONFT-MAURY *et coll* 1964).

The oxygen enhancement ratio for high energy protons is close to what has been found for other low LET radiation (LARSSON & KIHILMAN 1960, LARSSON & STENSON 1965).

Dose distribution obtainable with high energy protons in radiotherapy

A number of tentative dose distributions for various tumour localizations were constructed in order to demonstrate possible applications of high energy protons in radiotherapy. The following criteria were applied in the selection of the fields: (1) the dose to critical organs must not exceed adopted tolerance levels, (2) the dose to the primary tumour volume should be tumouricidal as judged by current standards, (3) the region susceptible of lymphatic spread should, if so considered appropriate, receive a tumouricidal dose and (4) the anticipated damage to normal tissue, and to critical organs in particular, should be as small as possible.

The resulting dose distributions are shown in Fig. 4. The low dose to critical organs, the low integral dose and the outstanding capacity of giving full tumouricidal dose to the primary tumour and to the regional lymphatic system without violating restrictions posed by tolerable integral doses or anticipated damage to critical organs should be noted. The applications of proton fields to tumours of superficial localization is not exemplified but it is obvious that for e.g. breast tumours the capacity of a total avoidance of lung and heart irradiation should be of clinical importance.

The individual dose distributions are commented on below.

Pharynx Because of their deep localization, their closeness to vulnerable organs such as the eyes and the medulla and their extremely high tendency of regional lymphatic spread nasopharyngeal tumours are one of the technically most difficult treatment objects in the head and neck region. The problems involved have been discussed in detail by e.g. LIDFMAN & MOULD (1968) and MILLION *et coll* (1963). Both groups recommend two opposing fields to the nasopharyngeal region and an anterior split field to the neck nodes. Such a dose distribution is easily achieved with high energy protons but a field arrangement as illustrated in Fig. 4 ought to have a higher probability of covering all lymph nodes. Bolus of rather intricate shape has to be applied in order to achieve the correct pen-

Table 1

Probability of regional (p_2) and distant (p_3) spread of various tumour types

Reference	Tumour localization	p	p	Comments
MERENDINO & MARK 1952	Oesophagus	0.40	0.32	Squamous cell carcinoma operation or autopsy material
TOMLINSON & WILSON 1943	Oesophagus	1.00		Squamous cell carcinoma autopsy material
BLOEDORN 1966	Bronchus	0.70	(0.85)	All histologic types operation material p estimated from frequency of blood vessel invasion
HORWITZ et coll 1963	Bronchus		0.80	All patients at admission
LARSSON & ROMANUS 1963	Cervix	0.08		
	Larynx	0.13		
	Larynx	0.15		
	Lingua	0.45		
	Hypopharynx	0.45		
	Floor of mouth	0.50		
	Tonsilla	0.75		
MILLION et coll 1963	Tonsilla	0.74		Unilateral spread $p=0.63$ bilateral spread $p=0.11$
	Base of tongue	0.77		Unilateral spread $p=0.47$ bilateral spread $p=0.35$
	Pharynx	0.90		Unilateral spread $p=0.39$ bilateral spread $p=0.51$
LEDERMAN 1961	Pharynx	0.70		
LENE 1942	Pharynx	0.53	0.29	
REIFFENTHAL 1967	Cervix uteri	0.19		Stage I
		0.32		Stage II
		0.53		Stage III
		0.67		Stage IV
NOTTMAYER 1964	Cervix uteri		0.02	Stage I from material with primary healing of primary tumour
			0.03	Stage II from material with primary healing of primary tumour
			0.09	Stage III from material with primary healing of primary tumour
			0.11	Stage IV from material with primary healing of primary tumour

quantitative study of the frequency of cases where a curative treatment is not given because of anticipated damage to critical organs due to limitations in the shaping of the radiation field would be of special interest in this context, since the major merit of high energy protons may be to reduce this frequency.

The integral dose has often been referred to as some kind of a figure of merit for comparing different techniques of dose administration. To our knowledge, it has not been possible, however, to demonstrate, on a patient material, the influence of the integral dose, as distinct from local dose, on the clinical result (cf e.g. KOHN et coll 1965). There is a tendency among radiotherapists, however, to prescribe a lowered tumour dose or an extended treatment period when the integral dose is extraordinarily high or when the patient is in a weak condition and hence would not tolerate the effect of, possibly, the integral dose. This would imply that a larger number of the patients could be curatively treated with a radiation type that gives a low integral dose. There appears however to be no way of assessing quantitatively, from literature data, the importance of this fact. If there is a natural defence mechanism against neoplastic cells (cf e.g. SUIT et coll 1967) its effectiveness might be correlated to the integral dose. Probably, the correlation is negative although the opposite cannot be excluded (SILVER et coll 1964).

Estimate of difference in cure rate between two therapy procedures

As the beneficial effects of high energy protons cannot be estimated directly from empirical findings, a simple tumour model was constructed as an aid in the estimation of the difference in cure rate that can be expected between two therapy methods of which one may be proton therapy. Such an estimate is, of course, necessary for the planning of a clinical trial since it determines the number of patients that one should be prepared to include in the trial.

For the sake of simplicity the possible tumour bearing sites in the patient are divided into three volumes, a primary one including the primary tumour in its full extension, a secondary one covering the regional metastases that can be included in the volume (or volumes) irradiated by full tumouricidal dose and a tertiary volume that cannot be curatively treated by radiotherapy. The model is based on the assumption that the following five conditions are necessary and sufficient for a therapeutic procedure to be curative: (1) all parts of the primary tissue that are within the irradiated volume must be controlled, (2) there shall be no primary tumour growth outside this volume, (3) all parts of the regional metastatic spread that are within the irradiated volume must be controlled, (4) there shall be no regional metastases outside this volume, and (5) there shall be no tertiary spread of tumour.

Table 3

Local cure rates of primary tumour ($\alpha_1\beta_1$) and regional metastasis ($\alpha_2\beta_2$) frequency of regional (p_2) and distant (p_3) spread and cure rate (Q) as calculated by the model formula. Data taken from Tables 1 and 2. ΔQ_1 and ΔQ_2 are calculated increases in Q if $\alpha_1\beta_1$ and $\alpha_2\beta_2$ respectively are increased by (1 given value)/2

Tumour local: at on	$\alpha\beta$	$\alpha\beta$	p	p	Q	ΔQ	ΔQ_2	Comments
Bronchus	0.35	0.69	0.70	0.85	0.04	0.04	0.01	
Nasopharynx	0.63	0.55	0.80	0.79	0.29	0.08	0.08	
Collum uteri	0.99	0.84	0.19	0.02	0.94	0.00	0.01	Stage I
	0.88	0.56	0.37	0.05	0.77	0.05	0.06	Stage II
	0.57	0.70	0.33	0.09	0.44	0.17	0.04	Stage III

that tumour growth in the primary and secondary volumes situated within the irradiated volume is controlled one finds the cure rate Q to be given by

$$Q = \alpha_1\beta_1(q + p_2 - \alpha_2\beta_2)q_3$$

where

$$q = 1 - p \text{ and } q_3 = 1 - p_3$$

Admittedly this is a very coarse model but it serves our purpose of demonstrating how a change in one treatment factor (e.g. dose distribution, radiation type, fractionation regime, oxygen tension) may influence the survival of a group of patients. The model has the merit of splitting the causes of treatment failure into factors depending on the clinical status of the patient, the dose distribution and the local treatment efficiency, but it neglects the interdependence of the local cure rate and the probability of local and distant spread as well as all negative effects of radiotherapy. The formula is easily extended to cover also cases where there are several secondary tumour volumes and of course α and β may as well as p and p_3 be taken to depend on the clinical stage of disease as expressed by e.g. the TNM classification system. Surgical treatment can also be included in the formula.

There are few exhaustive reports in the literature on the patient's clinical status with respect to actual tumour spread at the time of admittance to the hospital. Of course there are good practical reasons for this lack of knowledge. For some tumour localizations there are however data that may be interpreted to give estimates of the tumour spread. A relevant part of this information is given in Table 1.

Data on the local cure rate of tumours at the primary site and in the lymphatic

Table 2

Local cure rate for primary tumour ($\alpha_1\beta_1$) and regional metastasis ($\alpha_2\beta_2$) for various tumour localizations

Reference	Tumour localization	$\alpha_1\beta_1$	$\alpha_2\beta_2$	Comments
BLOEDORN 1966	Bronchus	0.35	0.77	At operation after pre-operative radiotherapy
BLOEDORN 1966	Bronchus	0.35	0.60	At operation after pre-operative radiotherapy regional metastasis verified histologically
SLIT et coll. 1967	Anterior tongue	0.85		Squamous cell carcinoma stage T1-T4 palliative cases excluded
	Floor of mouth	0.90		Squamous cell carcinoma stage T1-T4 palliative cases excluded
	Pharyngeal wall	0.73		Squamous cell carcinoma stage T1-T4 palliative cases excluded
	Hypopharynx	0.70		Squamous cell carcinoma stage T1-T4 palliative cases excluded
	Larynx	0.88		Squamous cell carcinoma stage T1-T4 palliative cases excluded
LENZ 1942	Nasopharynx	0.63		
PERRY et coll. 1966	Metastasis in lymph nodes of neck		0.60	Primary tumour controlled
			0.50	Primary tumour partially controlled
RUTLEDGE 1966	Collum uteri		(0.84)*	Stage I after radiotherapy at operation
			(0.56)*	Stage II after radiotherapy at operation
			(0.70)*	Stage III after radiotherapy at operation
KOTTMEIER 1964	Collum uteri	0.99		Stage I
		0.88		Stage II
		0.57		Stage III

*Figures obtained from data on frequency of patients without regional metastasis after pre-operative radiotherapy and frequency of patients with metastasis at admittance to hospital given in Table 1

With this assumption and denoting by p the probability that the patient on admission has regional spread and by p_2 that he has distant spread by α_1 and α_2 the probabilities that all tumour cells in the primary and secondary volumes respectively, are within the irradiated volume and by β_1 and β the probability

Table 3

Local cure rates of primary tumour ($\alpha_1\beta_1$) and regional metastases ($\alpha_2\beta_2$) frequency of regional (p_1) and distant (p_2) spread and cure rate (Q) as calculated by the model formula. Data taken from Tables 1 and 2. ΔQ_1 and ΔQ_2 are calculated increases in Q if $\alpha_1\beta_1$ and $\alpha_2\beta_2$ respectively are increased by (1 given value)/2

Tumour localization	$\alpha\beta$	$\alpha\beta$	p	p	Q	ΔQ	ΔQ_1	Comments
Bronchus	0.35	0.69	0.70	0.85	0.04	0.04	0.01	
Nasopharynx	0.63	0.55	0.80	0.90	0.09	0.08	0.08	
Cervix uteri	0.99	0.84	0.19	0.07	0.94	0.00	0.01	Stage I
	0.88	0.56	0.32	0.05	0.72	0.05	0.06	Stage II
	0.57	0.70	0.53	0.09	0.44	0.17	0.04	Stage III

that tumour growth in the primary and secondary volumes situated within the irradiated volume is controlled one finds the cure rate, Q to be given by

$$Q = \alpha_1\beta_1(q + p_2\alpha\beta)q_2$$

where

$$q = 1 - p_1 \text{ and } q_2 = 1 - p_3$$

Admittedly this is a very coarse model but it serves our purpose of demonstrating how a change in one treatment factor (e.g. dose distribution, radiation type, fractionation regime, oxygen tension) may influence the survival of a group of patients. The model has the merit of splitting the causes of treatment failure into factors depending on the clinical status of the patient, the dose distribution and the local treatment efficiency, but it neglects the interdependence of the local cure rate and the probability of local and distant spread as well as all negative effects of radiotherapy. The formula is easily extended to cover also cases where there are several secondary tumour volumes and of course α and β may, as well as p_1 and p_3 be taken to depend on the clinical stage of disease as expressed by e.g. the TNM classification system. Surgical treatment can also be included in the formula.

There are few exhaustive reports in the literature on the patient's clinical status with respect to actual tumour spread at the time of admittance to the hospital. Of course there are good practical reasons for this lack of knowledge. For some tumour localizations there are however data that may be interpreted to give estimates of the tumour spread. A relevant part of this information is given in Table 1.

Data on the local cure rate of tumours at the primary site and in the lymphatic

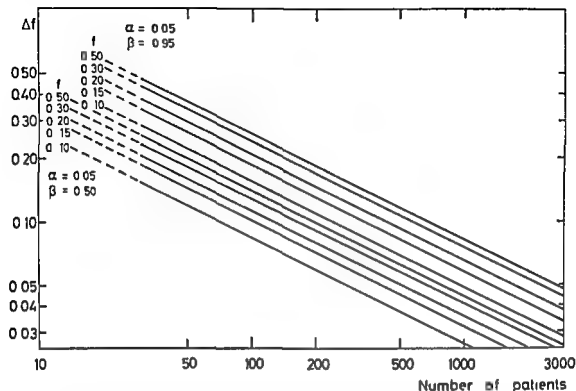


Fig. 5. Diagram showing the relation between the expected difference in cure rate (or similar measure) Δf and the number of patients that should be included in each group of a clinical trial in order to have a 95% ($\beta = 0.95$) and 50% ($\beta = 0.50$) probability for a significant ($\alpha = 0.05$) result. The parameter f is the mean result of the two treatment types.

nodes are also hard to obtain from the literature. This may partly be due to the fact that the local cure rate is difficult to define and hence hard to assess. (Similar arguments can, of course, be raised against the whole model, but it is hoped that the lack of stringency is of minor importance in the present context in which only crude estimates are aimed at.) From some reported data local cure rates for primary and sometimes also secondary tumours of various location and histologic type can be inferred. These are given in Table 2.

Also when the information is taken from several sources there are very few tumour localizations for which figures can be given for both the tumour spread and the local cure rate. These cases are collected in Table 3, in which also the cure rate obtained by the formula is given, as well as the increase in cure rate that can be expected for half the maximum possible increase in either $a_1\beta_1$ or $a_2\beta_2$, i.e. an increase by $0.5(1/a_1\beta_1)$ and $0.5(1/a_2\beta_2)$, respectively. The high values of Q and ΔQ_1 in Table 3 for stage III of cervical cancer can be explained by the fact that the p_3 value is derived from a material where healing of the primary tumour had occurred.

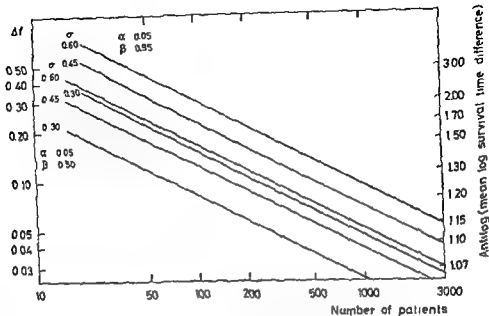


Fig 6 Diagram showing the relation between the expected difference in mean log survival time and the number of patients that should be included in each group of a clinical trial in order to have a 95% ($\beta = 0.95$) and 50% ($\beta = 0.50$) probability of finding a significant ($\alpha = 0.05$) difference in the results. The parameter σ is the standard deviation of the log mean survival time distribution.

No data were found that allow an estimate of σ and β separately. Both factors are retained in the formula since it is believed that they may be of at least theoretical interest.

The data indicate that an increase in Q by 0.10 is a very successful result and that often a much smaller increase must be expected. It may well be argued that this is a meagre result of the work spent on the model calculations. These are nonetheless retained here since the model, at least to us, seems to indicate a route by which, when the proper basic knowledge has been accumulated, it may be possible to estimate, with some accuracy, the merits of a change in treatment procedure.

Statistical procedures

Using the non-central F distribution given by e.g. PEARSON & HARTLEY (1951) it is easy to find, with some obvious simplifying assumptions, the relation between the expected difference in survival rate and the necessary number of

Table 4

Results of clinical trials p = reported level of significance e, f = reported results for the two treatments and $1, f$ = difference that would have been announced as significant ($p \approx 0.05$) with a probability of 0.50 and 0.95

Tumour localization and treatment type: treatment common to both groups is given before treatment different is separated by /	Measure of result	Mean number of patients in the two groups
<i>Bronchial carcinoma</i>		
Surgery vs MV roentgen	1 years	29
		19
Radiotherapy/surgery	Mean	72
Roentgen 8 MV 3 000 rad/4 000 rad	Mean	1
	2 years	
Pneumonectomy without/with roentgen	3 years	101
Radiotherapy split course/routine	1 year	15
^{60}Co gamma ray/3 atm O_2	13 months	25
^{60}Co gamma ray chlorambucil/placebo	Median	12
Roentgen synkavit + O_2 /synkavit	Mean log	68
	/compound 28	57
	/ O_2	56
Roentgen synkavit + O_2 /synkavit		45
	/compound 28	37
	/ O_2	38
Roentgen synkavit/no sensitizer	Mean	42
Roentgen + synkavit intravenous/intramuscular	Mean	28
Roentgen 5 FU/no sensitizer	Mean	13
Roentgen split course cytosine/5 FU	Mean	7
<i>Buccal carcinoma</i>		
^{60}Co gamma ray synkavit/no sensitizer	Local healing	47
<i>Lingual carcinoma</i>		
^{60}Co gamma ray synkavit/no sensitizer	Local healing	18
<i>Tumour metastasis in neck region</i>		
Neck dissection: 18 MeV eV pre operatively/no radiotherapy	Local healing	61
<i>Bladder carcinoma</i>		
^{60}Co gamma ray air/3 atm O_2	13 months	20
<i>Cancer mammae</i>		
Surgery with/without immediate radiotherapy	5 years	355
		370
Extended radical mastectomy with/without roentgen	5 years	153
		89
<i>Cancer cervix uteri</i>		
Radium after/before roentgen	5 years	24
		87

Table 4 (cont.)

P	f	df		Comments	Ref r ence
		0.50	0.95		
—	0.23/0.07	0.19	0.35	Operable carcinoma all histologic types	1
0.05	0.30/0.06			Operable squamous cell carcinoma	
0.05	256d/190d			Oat celled carcinoma	2
0.05	9.3m/6.0m			Inoperable anaplastic carcinoma	3
—	0.06/0.00				
—	0.36/0.33	0.13	0.25		4
—	0.20/0.07	0.3	0.5	Inoperable squamous cell carcinoma	5
—	0.25/0.20	0.23	0.46		6
0.001	7.4m/5.0m				7
—	0.69/0.67	0.13	0.27	Inoperable tumours males	8
—	/0.65	0.13	0.24		
—	/0.63	0.13	0.24		
—	0.73/0.66	0.14	0.27	Histological verified tumours	
—	/0.68	0.15	0.29		
0.05	/0.57	0.15	0.29		
0.001	8.7m/3.8m				9
0.01	11m/5.9m				
0.05	17m/6.2m			Inoperable carcinoma	10
—	6.8m/5.8m				
0.1	0.41/0.70	0.19	0.37	Resolution and no recurrence	11
—	0.23/0.09	0.2	0.4	Resolution and no recurrence	
0.05	0.13/0.33	0.15	0.29	No recurrence within 1 year	12
	0.33/0.17	0.3	0.5		6
	0.45/0.44	0.07	0.14	Irradiation of operation flap and axilla	13
	0.43/0.39	0.07	0.13	Irradiation of regional lymph nodes	
	0.64/0.11	0.11	0.21	All operable cases	14
—	0.1/0.71	0.13	0.25	Clinical stage I	
	0.35/0.44	0.3	0.5	Clinical stage II	
0.07	0.45/0.33	0.15	0.27	Clinical stage III	15

Table 4 (cont.)

Tumour localization and treatment type treatment common to both groups is given before, treatment different is separated by /	Measure of results	Mean number of patients in the two groups
Radium with/without roentgen	3 years	187
Radium and external irradiation kilovoltage/megavoltage	3 years	147
Radiotherapy with/without lymphadenectomy	3 years	33
		39
		27
		26
		30
⁶⁰ Co gamma conventional/grid technique	1 year	13
Cancer of pharynx larynx and bladder		
Roentgen progressive dose/even dose	5 years	151
Cancer of upper digestive and respiratory passage		
Roentgen 4 MV 1 atm O ₂ /air	6 months	13

References

- MORRISON et coll 1963
- DELFY 1967
- DELFY 1966
- PARKERSON & RUSSEL 1962
- LEVITT et coll 1967
- CADF & McLWEN 1967
- HOWITZ et coll 1963
- MITCHELL & HAYBITTLE 1965
- MITCHELL 1963
- GOLLIN et coll 1964

patients in each group of a clinical trial for a 95 % or 50 % probability of a result that is significant on the 5 % level (cf Fig 5).

When the aim of a radiotherapeutic procedure is palliation, the survival time might be a relevant measure. BOAC (1948, 1960) and others (e.g. KAUFMAN 1966, LEONARDUZZI et coll 1967) have demonstrated that for fatal patients the logarithms of the survival times are approximately normally distributed. The standard deviation of the distribution is about 0.45 when logarithms to the base of 10 are used. In Fig 6 are given curves which correlate the necessary number of patients for 95 % and 50 % probability of a significant result and the expected difference in mean log survival time. The curves are based on the non central Γ distribution.

Re assessment of clinical trials

In a large number of the fully analyzed clinical trials the verdict has read 'difference not significant'. Since this statement tells little about the magnitude

Table 4 (cont)

II	f	Δf		Comments	Reference
		0.50	0.95		
—	0.67/0.59	0.10	0.19	Clinical stage I and II	16
—	0.68/0.68	0.10	0.20	Clinical stage I and II	
—	0.93/0.86	0.14	0.26	Clinical stage I	17
—	0.89/0.77	0.14	0.25	Clinical stage IIa	
—	0.62/0.54	0.25	0.50	Clinical stage IIb	
—	0.33/0.54	0.24	0.50	Clinical stage IIIa	
—	0.63/0.54	0.25	0.50	Clinical stage IIIb	
0.007	0.69/0.07			Clinical stage III and IV	18
—	0.35/0.33	0.10	0.20		19
—	0.80/0.64			$4 \times 725 \text{ rad}/4 \times 775 \text{ rad for } O_2/\text{air}$	20

- | | |
|--------------------------------|---------------------------|
| 11 SHANTA & KRISHNAMURTHI 1964 | 16 PATERSON & RUSSEL 1964 |
| 12 HENSCHKE et coll 1964 | 17 RUTLEDGE et coll 1965 |
| 13 PATERSON & RUSSEL 1959 | 18 DEED & BARNHARD 1964 |
| 14 KAAE & JOHANSEN 1967 | 19 PATERSON & RUSSEL 1962 |
| 15 PATERSON & RUSSEL 1962 | 20 VAN DEN BRENK 1968 |

of any real difference that could have remained undetected or that should have been detected with a fair probability we have collected in Table 4 the clinical trials in radiotherapy that we could obtain reports on. From the number of patients entering the trial the difference (Δf) that should have given a significant difference (on the 5% level) in 50% and 95% of the trials can be read from Figs 5 and 6 for those trials in which the measure was survival rate or mean log survival time. Most of the authors split the patient material in various subgroups according to age, clinical status and histologic type of tumour and hence it has not been possible to include all reported results in the table. We have tried to limit the presentation to those data which we believe illustrate best the outcome of the trial.

It was found that in most of the trials differences as large as 0.20 in survival rate could not be excluded (the criterion for exclusion being a probability of less than 5% for a non significant difference in results) and in no case could a 50% chance for detection of a 0.05 difference be attained.

There is only one case (MORRISON *et coll* 1963) in which a significant

difference in cure rate after a reasonable length of time has been announced. The reported difference in 4 year survival is 0.14. In two other cases (MORRISON et coll., PATERSON & RUSSELL 1962) an almost significant difference has been announced. The differences in survival rate were 0.16 and 0.15, respectively. It is concluded that the estimate of difference in cure rate between two therapy protocols arrived at, (see p. 13) is valid, remembering the fact that the habit of contrasting the patient material in several different ways increases the risk for errors of the second type, and taking into account also the differences found in not significant trials. In fact, it can be demonstrated with a χ^2 test that the cohort of clinical trials is not at variance with the null hypothesis that in no trial was there any difference between the two regimes.

There is only one trial in which the measure was the mean log survival time. A significant difference was announced for one of the contrasted pairs of protocols. The difference in mean log survival time was in this case 0.21. It is meaningless to draw any conclusion from this single case as to what difference in mean log survival time could be expected in other trials.

The information from the remaining clinical trials collected in Table 4 does not lend itself to the type of analysis attempted here.

Conclusions

With a blunt formulation it may be stated that future technical advancements and changes in techniques are not likely to bring about, in one single step, a substantial improvement in the clinical result of radiotherapy. The complexity of tumour therapy, the access to modern apparatus and the body of information, required through clinical experience and substantiated by follow up studies act in concert to this end. Rigid statistical methods are thus necessary if the small gains in survival rate that can be hoped for shall be detected. Including factorial designs as being much too unwieldy, though perhaps more efficient, only clinical trials remain.

It seems to be generally agreed that clinical trials are ethically allowed only when the investigator does not know beforehand which of the two protocols gives the better result. Follow up studies from various radiotherapy clinics indicate that for well classified patient materials the treatment results are remarkably similar irrespective of environmental factors and the admittedly marginal differences in treatment techniques. This would imply that the uncertainty in the estimate of clinical result of a conventional protocol is low, say below 0.10 in survival rate. The uncertainty in the estimate for the contrasted protocol should be of the same magnitude. This would imply that the very method of clinical trial, at the present stage of development, restricts the likely difference in clinical

result to values similar to those indicated by the calculations with the tumour therapy model and by the outcome of the analysis of published clinical trials

Taking into account the complex interplay between all the factors controlled by the therapist essential improvements should still be achievable. Each step necessitates however a number of patients often of the order of 1 000 or more. Only at very large therapeutic centers or through inter clinical cooperation meticulously planned and carried out can these numbers be collected within a reasonable length of time.

As to high energy protons applied on a moderate scale it seems obvious that for patients that can be given full tumour dose with modern conventional equipment the possible merits will be very hard or indeed impossible to assess. There may remain however a sizable class of patients to which a homogeneous full tumour dose can be given only with protons and not with common types of radiation due to the geometrical differences of the radiation fields. Were a trial confined to such a restricted group of patients the difference in clinical result might be quite large and it may in fact be questioned whether such a trial would be ethical. The literature gives, however very little guidance in these matters as the palliatively treated patients often are excluded in the follow up studies or referred to one single group.

The discussion of clinical result has been confined mainly to the cure rate this measure being the best available today or at least the one most widely accepted. It does not however take the untoward effects of radiotherapy into proper account but probably better so than the other two measures discussed namely the local cure rate and the mean log survival time. The latter measure furthermore is less well suited when there is a substantial cure rate.

If other valid and more sensitive measures of both negative and positive effects were available the rate of progress might be considerably speeded up.

SUMMARY

The physical radiobiologic and radiotherapeutic properties of high energy protons are summarized and tentative dose distributions for the treatment of tumours in the pharynx, oesophagus, lung, urinary bladder and uterus constructed. The dose distribution conforms well with established criteria but the beneficial effects to be gained from them cannot be assessed quantitatively. A crude tumour model indicates that a 0.10 difference in cure rate between two therapy protocols is more than can be expected in most cases. Reassessment of results from clinical trials seems to confirm this estimate. Other measures of the clinical efficiency such as the mean log survival time and the local response may be more sensitive but are probably not good in a series of effects searched for. The conclusion is that of the order of 1 000 patients should be entered in each group of a clinical trial if the difference that can be expected is to be demonstrated significantly with a fair probability.

difference in cure rate after a reasonable length of time has been announced. The reported difference in 4 year survival is 0.14. In two other cases (MORRISON *et coll.*, PATERSON & RUSSELL 1962) an almost significant difference has been announced. The differences in survival rate were 0.16 and 0.15, respectively. It was concluded that the estimate of difference in cure rate between two therapy protocols arrived at, (see p. 13) is valid, remembering the fact that the habit of contrasting the patient material in several different ways increases the risk for errors of the second type, and taking into account also the differences found in not significant trials. In fact, it can be demonstrated with a χ^2 test that the cohort of clinical trials is not at variance with the null hypothesis that in no trial was there any difference between the two regimes.

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- DEELEY T A clinical trial to compare two different tumour dose levels in the treatment of advanced carcinoma of the bronchus *Clin Radiol* 17 (1966) 299
- The treatment of carcinoma of the bronchus *Int J Radiol* 40 (1967) 801
- FALKNER S FORS B LARSSON B et coll Pilot study on proton irradiation of human carcinoma *Acta radiol* 58 (1962) 33
- FORSS B LARSSON B LINDELL A et coll Further studies on the effect of high energy protons on human genital carcinoma *Acta radiol Ther Phys Biol* 2 (1964) 384
- GOLLIN F F ANSFIELD F J and VERMUND H Clinical studies of combined chemotherapy and irradiation in inoperable bronchogenic carcinoma *Amer Roentgenol* 92 (1964) 88
- GRAFFMAN H and JUNG B Behaviour of ^{14}C and ^{15}O induced in mouse by 175 MeV protons European Office of Aerospace Research U S Air Force Sci Rep No 6 (1967) of Contract No AF 61 (032)—740
- HILGESSON R JUNG B and NOHRMAN H A High-energy protons in postoperative treatment of glioblastoma multiforme *In Progress in Radiology Proc XI Intern Congress of Radiology Rome 1965*
- JUNG B NOHRMAN H A and BERGSTRÖM R Supplementary treatment of nasopharyngeal tumours with high energy protons *Acta radiol Ther Phys Biol* 3 (1967) 461
- — LARSSON B et coll High-energy protons in radiotherapy Recent developments *In Progress in Radiology Proc XI Intern Congress of Radiology Rome 1965*
- HALLER I B and JUNG B Radionuclides produced in man by high energy protons European Office of Aerospace Research U S Air Force Sci Rep No 1 (1963) of Contract No AF 61 (032)—740
- HILGESSON R U K FRANZELL E L HILARIN B S et coll Local recurrences after radical neck dissection with and without preoperative X ray therapy *Amer J Roentgenol* 82 (1964) 331
- HORWITZ H WRIGHT T L PERRY H and DARETT C M Suppressive chemotherapy in bronchogenic carcinoma A randomized prospective clinical trial *Amer J Roentgenol* 93 (1965) 615
- ICR/ICRL Report of the RBE Committee *High Phys* 9 (1963) 356
- JOHANSSON K J and LARSSON H Effects of high-energy protons and ^{60}Co -gamma radiation on the incorporation of ^{14}C -thymidine into intestinal and splenic DNA To be published
- JUNG B Radionuclide production and effects of nuclear reactions on dose from 193 MeV protons stopped in tissue European Office of Aerospace Research U S Air Force Sci Rep No 2 (1963) of Contract No AF 61 (032)—740
- KAAK S and JOHANSEN H Breast cancer Five year results Two random series of simple mastectomy with postoperative irradiation versus extended radical mastectomy *Amer J Roentgenol* 87 (1962) 87
- KARLSON B Methoden zur Berechnung und Erzielung einiger für die Tiefentherapie mit Protonen günstigen Dosisverteilungen *Strahlentherapie* 124 (1964) 481
- KRAFTMAN H Allgemeine Gesichtspunkte zur Darstellung von Behandlungsergebnissen bei tödlich verlaufenden Erkrankungen *Strahlentherapie* 130 (1966) 81
- Ein einfaches Verfahren zur Auswertung von Überlebenskurven bei tödlich verlaufenden Erkrankungen *Strahlentherapie* 130 (1966) 509
- KJELLBERG H A Personal communication (1967)
- FIELD J W McMEEL J W and SWET W H Bragg peak pituitary destruction for diabetic retinopathy *Proc III Intern Congr Neurological Surgery Copenhagen 1965*
- KOHLER A M PRISTON W M and SWET W H Intracranial lesions made by the

ZUSAMMENFASSUNG

Die physikalischen, radiobiologischen und radiotherapeutischen Eigenschaften hochenergetischer Protonen werden zusammengefasst und versuchsweise Dosisverteilungen für die Behandlung von Tumoren des Pharynx, des Oesophagus, der Lungen, der Blase und des Uterus konstruiert. Die Dosisverteilungen stimmen gut mit den aufgestellten Kriterien überein, aber die vorteilhaften Effekte, die daraus gewonnen werden können, nicht quantitativ eingeschätzt werden. Ein grobes Tumormodell deutet darauf hin, dass eine 0.10 Differenz der Heilungsrate zwischen zwei Therapieprotokollen grosser ist, als sie in den meisten Fällen erwartet werden kann. Die Wertung der Resultate klinischer Versuche scheint diese Schätzung zu bestätigen. Andere Masse der klinischen Wirkung wie die mittlere logarithmische Überlebenszeit und die lokale Reaktion mögen empfindlicher sein, sind aber wahrscheinlich keine guten Masse des gesuchten Effekts. Daraus folgert, dass jede klinische Versuchsgruppe die Grossenordnung von tausend Patienten umfassen sollte, wenn man die Signifikanz der zu erwartenden Differenz mit annehmbarer Wahrscheinlichkeit nachweisen will.

RÉSUMÉ

Les auteurs résument les propriétés physiques, radio-biologiques et radiothérapeutiques des protons de haute énergie et construisent à titre d'essai les distributions de dose pour le traitement des tumeurs du pharynx, de l'oesophage, des poumons, de la vessie et de l'utérus. La distribution de dose concorde bien avec les critères établis mais l'amélioration qui en résulte ne peut être jugée quantitativement. Un modèle rudimentaire de tumeur montre qu'une différence de 0.10 dans la dose de guérison entre deux protocoles de traitement est plus qu'on ne peut attendre de la plupart des cas. L'étude des résultats des essais cliniques paraît confirmer cette estimation. D'autres mesures de l'efficacité clinique telle que le logarithme de la moyenne de la durée de survie et la réponse locale peuvent être plus sensibles mais ne sont probablement pas de bonnes mesures de l'effet étudié. La conclusion de cette étude est que chaque groupe d'essais cliniques devrait comprendre environ 1 000 malades pour qu'on puisse mettre en évidence avec une bonne probabilité la différence prévue.

REFERENCES

- BROFDORN I. G. (a) Rationale and benefit of prooperative irradiation in lung cancer. *J. Amer. med. Ass.* 9 (1966) 310.
 — (b) Lung. In: *Textbook of radiotherapy*, p. 402. Edited by G. H. Fletcher, Lea & Febiger, Philadelphia, 1966.
 BOAC J. W. The presentation and analysis of the results of radiotherapy. *Brit. J. Radiol.* 21 (1948) 128 and 189.
 — Statistical aspects of planning clinical experiments in cancer therapy. *Acta radiol.* 51 (1960) 289.
 BONET MAURY P., BAARLI J., KAHN T. et coll. Efficacité biologique relative sur la souris et d'autres organismes de protons et des électrons de haute énergie. *Proc. IAEA Conference*, Upton N. Y. 1964.
 CADE I. S. and McFARLANE J. H. Megavoltage radiotherapy in hyperbaric oxygen. *Cancer* 20 (1967) 817.
 DEED E. I. and BARNHARD H. J. Focused grid telecobalt therapy in advanced epidermoid carcinoma of the cervix. *Radiology* 83 (1964) 228.

- PATERSON R. Clinical trials in malignant disease Part I Principles of random selection *J Fac Radiol (Lond)* 9 (1958) 80
- and RUSSEL M. II. Clinical trials in malignant disease Part III Breast cancer Evaluation of post-operative radiotherapy *Ibid* 10 (1959) 175
- Clinical trials in malignant disease Part IV Lung cancer Value of post-operative radiotherapy *Clin Radiol* 13 (1962) 141
- Clinical trials in malignant disease Part V Study in X ray technique *Ibid* 13 (1962) 207
- Clinical trials in malignant disease Part VI Cancer of the cervix uteri Is X ray therapy more effective given before or after radium *Ibid* 13 (1962) 313
- Clinical trials in cervix cancer *Rev Inst Nat Cancer* 16 (1964) 425
- PEARSON E. S. and HARTLEY H. O. Charts of the power function for analysis of variance test derived from the non-central F-distribution *Biometrika* 38 (1951) 112
- PERRY H. CHU F. C. H. KITAGAWA T. et coll. Clinical evaluation of the treatment of advanced head and neck cancer with high energy electron beams *Cancer* 19 (1966) 1081
- PIERQUIN B. WAMBERSIE A. and TUBIANA M. Cancer of the thoracic oesophagus Two series of patients treated by 33 MeV betatron *Brit J Radiol* 39 (1966) 189
- RIFKENSCHUL D. Das Lymphknotenproblem beim Carcinoma colli uteri und die Lymph-irradiation pelvis Urban und Schwarzenberg München 1967
- RUTLEDGE F. V. The role of surgery in irradiation therapy for cervical cancer *In* Textbook of radiotherapy p. 474 Edited by G. H. Fletcher Lea & Febiger Philadelphia 1966
- FLETCHER G. H. and MACDONALD E. J. Pelvic lymphadenectomy as an adjunct to radiation therapy in treatment for cancer of the cervix *Amer J Roentgenol* 93 (1965) 607
- SIANTA V. and KRISHNAMURTHI S. Evaluation of Synkavit-sensitized radiation therapy in radioresistant oral carcinomas *Radiology* 83 (1964) 687
- SIKOV M. R. COOK B. B. and LOFSTROM J. E. Effect of total body irradiation on the response of transplanted mouse tumours to fractionated local radiotherapy *Radiology* 83 (1964) 807
- STÉANSON S. Weight changes and mortality of rats after abdominal proton and roentgen irradiation *Acta radiol Ther Phys Biol* 8 (1969) 423
- STEWART J. R. COHN K. E. FAJARDO L. F. et coll. Radiation induced heart disease A study of 25 patients *Radiology* 89 (1967) 307
- SUIT H. WETT R. and LINDBERG H. Analysis of tumour recurrence times *Radiology* 88 (1967) 311
- TOBIAS C. V. Personal communication (1967)
- LAWRENCE J. H. LYMAN J. et coll. Progress report on pituitary irradiation *In* Response of the nervous system to ionizing radiation p. 19 Edited by T. J. Haley and R. S. Snyder Little Brown and Co Boston 1964
- TOMLINSON W. J. and WILSON L. A. Esophageal carcinoma in British West Indian and Pan American Negro a study of the incidence etiological factors and pathological anatomy in 50 cases *Arch Path Chic* 39—40 (1945) 79
- VAN DEN BREK H. A. II. Hyperbaric oxygen in radiation therapy An investigation of dose effect relationships in tumour response and tissue damage *Amer J Roentgenol* 108 (1968) 8
- ZERRY C. D. and KINSLEY W. E. Current — dose conversion factors for beams of high energy protons *ORNL-TM* 1038 (1965)

- Bragg peak of a proton beam *In* Response of the nervous system to ionizing radiation p 36 Edited by T J Haley and R S Snider Little Brown and Co Boston 1966
- SWEET W H, PRESTON W M and KOEHLER A M The Bragg peak of a proton beam in intracranial therapy of tumours *Trans Amer neurol Ass* 87 (1962), 216
- KOHN H I, BAILAR J C and ZIPPIN C Radiation therapy of the cervix Its late effect on the lifespan as a function of regional dose *J Nat Cancer Inst* 34 (1965) 345
- KOTTMEIER H L Surgical and radiation treatment of carcinoma of the uterine cervix *Acta obstet gynec scand* 43 (1964) Suppl No 2
- LARSSON B Pre therapeutic physical experiments with high energy protons *Brit J Radiol* 34 (1961), 143
- Radiological properties of beams of high energy protons *Radiat Res Suppl* 7 (1967) 304
- and KIHLMAN B A Chromosome aberrations following irradiation with high energy protons and their secondary radiation A study on dose distribution and biological efficiency using root tips of *Vicia faba* and *Allium cepa* *Int J Radiat Biol* 2 (1960) 8
- and STENSON S Reduction of radiation damage to the intestinal mucous membrane through local hypoxia *Nature* 205 (1965) 364
- LARSSON L G och ROMANUS R Tumorer på huden (In Swedish) *In* Tumorsjukdomar p 310 Edited by S Feigenberg E Poppe and R Romanus Almqvist & Wiksell Stockholm 1963
- LEDERMAN M Cancer of nasopharynx Its natural history and treatment Charles C Thomas Springfield Illinois 1961
- and MOULD R F Radiation treatment of cancer of the pharynx With special reference to telecobalt therapy *Brit J Radiol* 41 (1968) 251
- JONES C H and MOULD R I Carcinoma of the oesophagus with special reference to the upper third *Brit J Radiol* 39 (1966) 193
- LENARDUZZI G, VALDAONI C and PISTOLESI G F Comparison of the results of treatment of 2 549 cases of malignant neoplasm with high energy radiation *In* Progress in Radiology Proc XI Intern Congr Radiology Rome 1965
- LENZ M Roentgen therapy of primary cancer of the nasopharynx *Amer J Roentgenol* 48 (1942) 816
- LEVITT S H, BOGARDUS C R and LADD G Split dose intensive radiation therapy in the treatment of advanced lung cancer A randomized study *Radiology* 80 (1967) 1159
- MERENDINO K A and MARK V H An analysis of hundred cases of squamous cell carcinoma of the oesophagus II With special reference to its theoretical curability *Surg Gynec Obstet* 9 (1952) 110
- MICHAÏLOV V Zur Frage der Herzmuskelschädigung bei Strahlentherapie im Gebiet des Thorax *Radiobiol Radiother (Berl)* 4 (1963) 423
- MILLION R R, FLETCHER G H and JESSE R H Evaluation of elective irradiation of the neck for squamous cell carcinoma of the nasopharynx tonsillar fossa and base of tongue *Radiology* 80 (1963) 973
- MITCHELL J S Clinical assessment of tetra sodium 2 methyl 1 4 naphthohydroquinone diphosphate as a radiosensitizer in the radiotherapy of malignant tumours *Brit J Cancer* 7 (1953) 313
- BRINKLEY D and HAYBITTLE J L Clinical trial of radiosensitizers including Synkavit and oxygen inhaled at atmospheric pressure *Acta radiol Ther Phys Biol* 3 (1965) 329
- MORRISON R, DEELEY T J and CLELAND W P The treatment of carcinoma of the bronchus A clinical trial to compare surgery and supervoltage radiotherapy *Lancet* 1963 I p 683

Table

Percentage output for various field areas normalized at the largest measured field area

Area in cm	4×4	5×5	7×7	10×10	15×15	20×20	25×25
Unit A	91.5	92.5	94.0	96.0	98.5	99.0	100
Unit B	74.0	83.0	88.5	93.5	97.5	99.0	100

metry. The accuracy of standard and sub-standard measuring equipment, coupled with the knowledge of essential factors determining the dose of radiation permits the calculation of physical data in radiation dosimetry to probably better than $\pm 3\%$. There is no doubt that this accuracy is maintained in good radiotherapy centers where in addition constant effort is being made to reduce to a minimum the phantom-patient differences and thus provide realistic patients dose distribution. However, it seems to us that many cobalt units tend to oversimplify or disregard various physical components and by doing so might easily commit errors approaching or even exceeding in value those associated with inherent clinical and biologic estimates.

It is a matter of common knowledge that the dosimeters for the measurement of high energy radiation are calibrated in equilibrium conditions by the National Bureau of Standards in U.S.A. It is also well known that the delivery of a cobalt 60 source is accompanied by manufacturer's certificate stating the strength of the source in rhm. However, it is often overlooked that the National Bureau of Standards calibrates the Victoreen ionizing chambers in a large radiation field of 500–600 cm and the manufacturers' data equally refer to the maximum field size of 25 cm \times 25 cm. Somehow, it is still traditionally believed that the fundamental calibration data refer to the area of 10 \times 10 cm; this simple misunderstanding might result in dose inaccuracy up to 7% on some cobalt units (see Table). In computing doses delivered to patients in cobalt teletherapy, surface backscatter factors are often disregarded. It is true that these factors are much lower than the ones used in conventional deep therapy. However, a look in the depth dose charts for cobalt 60 will show that the backscatter factor reaches the value 4.6% for a field size of 20 cm \times 20 cm. Large fields are used quite often and form an important part of our non-conventional techniques; there is no reason to neglect a well-established and calculated correction factor in cobalt dosimetry.

The distribution of the radiation beam across the irradiated field cannot be anticipated but has to be measured in conditions of actual irradiation patterns. Furthermore, these measurements should be carried out through different depths

DOSIMETRY OF IRREGULAR FIELDS IN COBALT 60 THERAPY

by

I M ARAL, D CARL M NISSEL and J SPIRA

In clinical work involving the use of ionizing radiation, the application of conventional or non conventional (irregular fields) in supervoltage therapy must be accompanied by an adequate knowledge of physical parameters. Unfortunately, considerable differences often exist between various centers in the methods of estimating the radiation dose and its distribution. Any attempt to compare results using identical treatment techniques is bound to fail unless it provides enough physical information to allow the reproducibility of the results and their evaluation. This is of paramount importance in the use of the so-called non conventional or irregular fields for which exact physical data form an important criterion of the new medical concepts.

The concept of absorbed dose and the almost universal use of rad in the exchange of medical information, contribute considerably to the understanding of differences in the biologic effects of radiation. However, a rad is as accurate as the measured roentgen; much smaller errors are committed by averaging in supervoltage therapy the rad-roentgen conversion factor between bone, fat and muscle tissues than by neglecting certain parameters in roentgen dose

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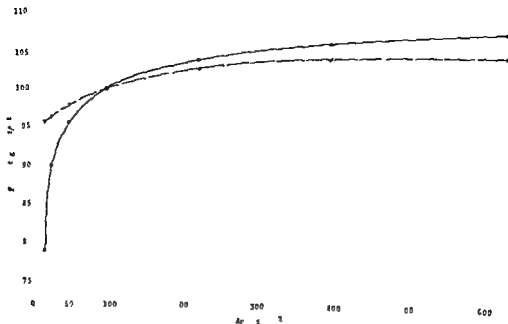


Fig 2 Output of rad at 100 cm versus field area normalized at 100 cm using cobalt 60 units A (—) and B (---)

Twelve Sievert chambers provided with additional lucite caps for electronic equilibrium were exposed in series of multiple consecutive exposures to a calibrated cobalt 60 beam and the sensitivity of each chamber in volts/roentgen was experimentally determined. The cobalt 60 doses were measured with the 25 R Victoreen condenser chamber provided with 4 mm lucite cap calibrated by National Bureau of Standards at the field size of 400–500 cm and exposed to the same fields at such a distance as to permit readings of approximately 10–12 roentgens representing the optimum linearity of the 25 R Victoreen chamber. The accuracy of the National Bureau of Standards standardization was $\pm 2\%$ and the reproducibility of the 25 R chamber was better than $\pm 1\%$.

The value of Adlux film in relative dosimetry of supervoltage radiation is well established and the techniques of film dosimetry in these regions have been discussed frequently in scientific publications. Finally in order to provide the most realistic approach to dose patient relationship in application of diversified fields of radiation an Alderson phantom was used consistently throughout our investigation. This phantom which has been fully described in professional literature allows measurement of radiation doses without errors resulting from the

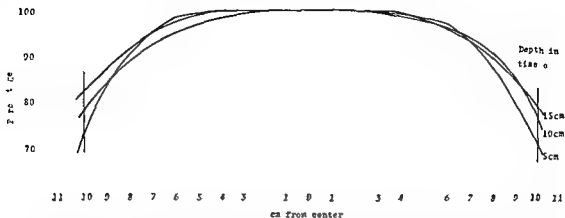


Fig. 1 Distribution of dose across the field at different depths with surface field area of 20 cm x 20 cm and using a cobalt 60 unit

in tissue and particularly through the central plane of the tumor. It can be shown that differences between the central dose and the dose at the edge of the irradiated field is a function of the depth of tissue: the across field becomes more uniform within the increased depth in tissue (Fig. 1). This brings to light another parameter which should not be neglected in the central dose calculations, frequently, rectangular fields are used with 2:1 or 2.5:1 or even 4:1 ratio of the side lengths. The use of equivalent square factors calculated by DAY for the determination of actual depth doses will eliminate a systematic error in patient dosimetry. Finally, the relationship between the output in air of cobalt 60 radiation under electron equilibrium conditions and the size of irradiated fields has to be experimentally established. We are fortunate to have in our institution two cobalt units which are similar in physical dimensions, source collimator distance, size and strength of the source, they differ considerably only in the construction of the collimating system. Measurements of radiation output in air versus the size of the irradiated field were performed on both units and the enclosed graph (Fig. 2) shows the very significant differences. It is clear that the change in radiation output with the field area is a function of a number of primary cobalt 60 photons scattered from the walls of the collimator system. The significant difference between the two units depends most probably on the type of the collimator and on source collimator geometry. We feel that accurate measurements of exposure field area factors should be performed on each cobalt 60 teletherapy unit and that these factors should be used in cobalt 60 dosimetry.

All the physical measurements pertaining to this investigation were performed using Sievert miniature ionization chambers in conjunction with the Baldwin Farmer electrometer, Adlux film was used for relative photographic dosimetry.

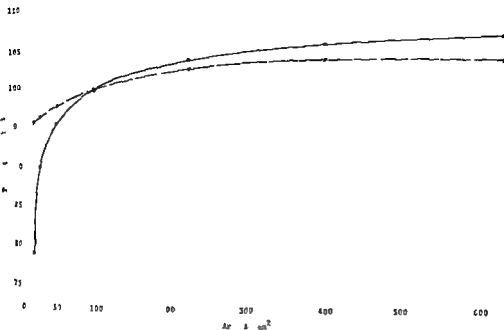


Fig 2 Output of radiation versus field area normalized at 100 cm² using cobalt 60 units A (— — —) and B (————)

Twelve Sievert chambers provided with additional lucite caps for electronic equilibrium were exposed in series of multiple consecutive exposures to a calibrated cobalt 60 beam and the sensitivity of each chamber in volts/roentgen was experimentally determined. The cobalt 60 doses were measured with the 25 R Victoreen condenser chamber provided with 4 mm lucite cap calibrated by National Bureau of Standards at the field size of 400—500 cm² and exposed to the same fields at such a distance as to permit readings of approximately 10—12 roentgens representing the optimum linearity of the 25 R Victoreen chamber. The accuracy of the National Bureau of Standards standardization was $\pm 2\%$ and the reproducibility of the 25 R chamber was better than $\pm 1\%$.

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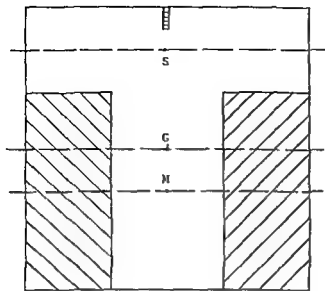


Fig. 3 Dose distribution in a T field

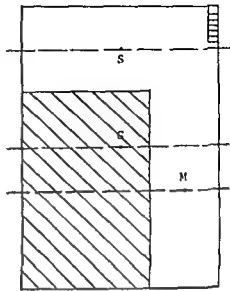


Fig. 4 Dose distribution in an L field

varying obliquity of the incident surface and from different densities of the tissues in question

Irregular portals have become quite commonplace in today's techniques for radical radiotherapeutic attacks on disease. These bizarre field shaping techniques are demanded because of the difficulty of accurately applying adjacent fields without running into overlap difficulties, or too widely separating fields, with accompanying problems of overdosage and underdosage.

We are accustomed in daily use to the application of the following unusual fields: a T shaped field in the treatment of malignancies of the mediastinum and supraclavicular regions, an L shaped field for unilateral use in similar clinical situations and in the abdomen, a mantle field for Hodgkin's disease as described by HAPLAN, and a diamond shaped field for pelvic irradiation. These are obtained by field shaping with lead blocks from rectangular primary areas.

Results

The T field (Fig. 3) In evaluation of the dose reaching the center of the mediastinal field at point M from the depth dose charts one must realize that the ap thickness of the patient at the center of the mediastinal field M, might be significantly greater than at the geometrical center point G of the 25 cm \times 25 cm irradiated field. This difference amounted to 5 cm in our phantom set up and the resulting measured dose was 10 % lower than the central depth

dose charts indicated for the position of point *G*. To correct this discrepancy one would measure the thickness of the patient through the two points *G* and *M* under identical respiratory condition and use the proper depth for *M* if significantly different.

In the center of the upper part of the *T* field at point *S* at 3.5 cm depth in tissue the radiation dose was found to be 6% less than the dose obtained from the standard depth dose charts for the particular depth and field area. It is significant to note that the dose at the irradiated depth of 3.5 cm in this part of the field drops continuously across the field. At 1 cm distance from the edge of the upper rectangle the dose was found to be 70% of the central dose indicated in the depth dose charts for this area and depth; the drop of the dose across the mentioned area is symmetrical.

The L field (Fig. 4) Results of our measurements show that the dose at point *S* representing the center at 3.5 cm depth of the supraclavicular part of the total irradiated field area of 17 cm \times 25 cm is 8% less than the dose charts for the specified conditions.

The dose at the center of the mediastinal field point *M* was found to be 6% less than the one calculated from the standard depth dose charts for unblocked total area of 17 cm \times 25 cm. It is interesting to note that identical measurements performed on our second cobalt unit II showed opposite results: the dose at point *M* was found to be 5% higher than the calculated from the depth dose charts. The reason for this discrepancy has been experimentally established: the distribution across the irradiated area in unit B shows a dip in the center of the field. At the present time we have no explanation why in this unit the dose in the center of a field is a few per cent lower than at the points some centimeters around the center. The mere fact, however, proves our previous contention that the physical parameters of each cobalt 60 unit cannot be taken for granted but have to be experimentally established.

Mantle field (Fig. 5) This field illustrates clearly the possibility of serious clinical errors resulting from the lack of actual dosimetric measurements in an elected treatment pattern. Due to the specific geometry of the field, the dose at the center of the mediastinal field at point *M* is equal to the dose calculated from the depth dose charts at the center of the 25 cm \times 25 cm diamond shaped field at point *G*. Furthermore, the dose at the center of the supraclavicular field point *S* at 3.5 cm depth is equal to the central depth dose calculated at the same depth for the 25 cm \times 25 cm field size. However, the dose change across the horizontal limb of the mantle is considerable. At the clinically important points *A* and *A'* of the axillary region, the dose was found to be only 70% of the calculated dose.

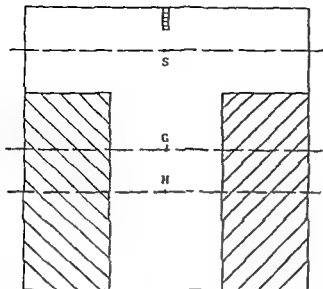


Fig 3 Dose distribution in a T field

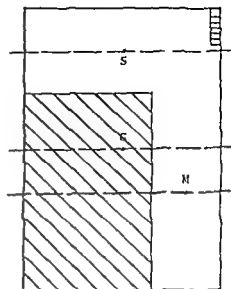


Fig 4 Dose distribution in an L field

varying obliquity of the incident surface and from different densities of the tissues in question.

Irregular portals have become quite commonplace in today's techniques for radical radiotherapeutic attacks on disease. These bizarre field shaping techniques are demanded because of the difficulty of accurately applying adjacent fields without running into overlap difficulties, or too widely separating fields with accompanying problems of overdosage and underdosage.

We are accustomed in daily use to the application of the following unusual fields: a T shaped field in the treatment of malignancies of the mediastinum and supraclavicular regions; an L shaped field for unilateral use in similar clinical situations and in the abdomen; a mantle field for Hodgkin's disease as described by KAPLAN, and a diamond shaped field for pelvic irradiation. These are obtained by field shaping with lead blocks from rectangular primary areas.

Results

The T field (Fig 3) In evaluation of the dose reaching the center of the mediastinal field at point *M* from the depth dose charts, one must realize that the ap thickness of the patient at the center of the mediastinal field *M* might be significantly greater than at the geometrical center point *G* of the 25 cm \times 25 cm irradiated field. This difference amounted to 3 cm in our phantom set up, and the resulting measured dose was 10 % lower than the central depth

boost up the dose to point *B*. If 4 cm blocks are used the gain at point *A* is almost 50 %. It is certainly of interest to know if in the evaluation of cures following the combined radium and external cobalt therapy this important figure does not really represent the optimal dosage rather than overdosage. For all the blocks used in this treatment pattern the dose to point *B* is 85 to 90 % of the dose delivered at the center of the unblocked field.

Conclusion

The concept of dosimetry in application of conventional or irregular fields in cobalt 60 therapy involves rigorous knowledge of physical parameters in determination of the radiation dose delivered to the patient. Some of the factors, i.e. the NBS calibration factor, backscatter and the area equivalence corrections have to be properly incorporated in the calculations; in others the output versus field area dependence and the dose determination across the irradiated area must be experimentally determined for each cobalt unit. In each treatment pattern the distribution of radiation throughout regions of anatomic importance was measured and discussed.

From the analysis of the results obtained it is apparent that if published depth dose tables are the sole source of dosage calculation clinically serious regions of over- or under dosage may result. From our measured data it is manifest that the dosage across large shaped fields and dosage under commonly used blocks cannot be predicted from the tables and should be determined individually for such fields and their respective blocks. Once incorporated into a library of dosimetry plans they may be freely applied wherever suitable.

SUMMARY

Output versus field size and the dose distribution with cobalt 60 units was experimentally measured for irregular fields commonly used in clinical radiotherapy. The results indicate that the dosage across large shaped fields and under commonly used blocks cannot be predicted from tables but should be determined individually for such fields and their respective blocks.

ZUSAMMENFASSUNG

Die Ausbeute im Verhältnis zur Feldgrösse und der Dosiserteilung mit Cobalt 60 Einheiten wurde experimentell für unregelmässige Felder wie sie gewöhnlich in der klinischen Radiotherapie verwendet werden, gemessen. Die Ergebnisse deuten darauf hin, dass die Dosierung über gross-formige Felder und bei den gewöhnlich verwendeten Blocken nicht aus Tabellen vorherbestimmt werden kann, sondern dass diese individuell für derartige Felder und ihre entsprechenden Blocke bestimmt werden sollte.

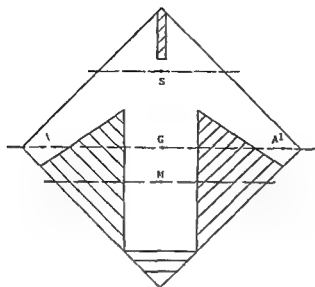


Fig 5 Dose distribution in a mantle field

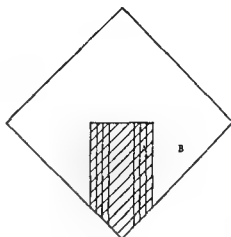


Fig 6 Dose distribution in a diamond shaped field

to the tumor. Therefore, we found it necessary to construct special compensators to spread the dose uniformly across the horizontal limb of the mantle field.

'Pelvic diamond' field (Fig 6) The diamond shaped field is a square field frequently $13\text{ cm} \times 13\text{ cm}$, turned 45° on its central axis perpendicular to the field surface. The irradiation field covers the obturator node, (the pelvic walls) the proximal one third of the vagina and the iliac nodes, while leaving the femoral heads outside the irradiated field. The diamond shaped field blocked along its midline to prevent over irradiation of the bladder and rectum, is widely applied following the insertion of radium system in the treatment of carcinoma of cervix and uterus. The data tabulated below represent the doses received at point A while using lead blocks of 5 HVL thickness and different widths of 2 cm, 3 cm, 4 cm and 5 cm measured on the skin of the patient. The measured doses are in percentage values of the dose received at the geometrical center of the unblocked field.

Width of block	Point A	Point B
2 cm	90	
3 cm	75	
4 cm	45	85—90
5 cm	25	

It will be noted that even with the maximum 5 cm width of the blocks used routinely, point A still receives around 25 % of the dose intended primarily to

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DOSE DISTRIBUTION FROM INTRACAVITARY RADIUM AND SUPPLEMENTARY EXTERNAL IRRADIATION WITH REGARD TO TOPOGRAPHY OF LYMPH NODES IN CARCINOMA OF THE UTERINE CERVIX

by

JAGMAR JOELSSON, ANDERS BACKSTROM JAN DIEHL and CURT LAGERGREN

Analysis of the results of radiation treatment of carcinoma of the cervix uteri at Radiumhemmet reveals that during the ten year period 1948—1957 the five year apparent recovery rate was considerably better than during the preceding decade. This applied particularly to clinical stages I and II a but less so to the stage II b and III. It appears that the improvement may be due at least in part to changes in intracavitary treatment policy with increased regard to the requirements of the individual patient. The principles of supplementary external irradiation to the parametrium remained the same.

Intracavitary treatment has not been altered since 1957 but the external therapy has been modified significantly. For purpose of comparison certain years have been taken as representative. For example during the period 1958—1963 conventional roentgen rays were given to the parametrium through four portals.

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RÉSUMÉ

Les auteurs ont fait des mesures expérimentales du débit en fonction des dimensions du champ et ont étudié la distribution des doses avec leur appareil de cobalt thérapeutique pour des champs irréguliers utilisés fréquemment en radiothérapie clinique. Leurs résultats montrent que la dose fournie par de grands champs et avec les blocs utilisés habituellement ne peut être déterminée d'après les tables et devrait être déterminée individuellement pour chaque champ et chaque bloc.

REFERENCES

- ARAL I M and SPIRA J. Practical dosimetry in cobalt 60 therapy. Presented at the meeting of the Radiological Society of North America in Chicago 1966
- NISSEL M, SPIRA J and GAIL D. Bizarre field dosimetry in clinical radiotherapy. Scientific exhibit presented at the meeting of the Radiological Society of North America in Chicago 1967
- BOTSTEIN C, SCHULZ R J and BIVON N. The use of a diamond shaped field in irradiation of pelvic organs particularly in carcinoma of the cervix uteri. *Amer J Roentgenol* 87 (1962) 44
- CLINICAL DOSIMETRY. Handbook No 87. National Bureau of Standards. Washington 1963
- DAY M J. The equivalent field method for x-ray dose determinations in rectangular fields. *Brit J Radiol* (1961) Suppl No 10
- KAPLAN H S. Role of intensive radiotherapy in the management of Hodgkin's disease. *Cancer* 19 (1966) 356
- TSIEN K C and ROBINS R. A comparison of a Cobalt 60 teletherapy unit and a 2 MeV Van de Graaf generator on the basis of physical measurements. *Radiology* 70 (1958) 486

ance from serial biopsies during treatment time (KOTTMEIER 1959) The sensitization and radiation responses in carcinoma of the cervix have been thoroughly investigated by HJELMGREN (1958) and RUBIO et coll (1965) All these studies have contributed greatly towards our present understanding of the subject even though the situation is still not clear

The present authors have consequently resorted to the physical aspects and have investigated the dose distribution in the pelvis from routine intracavitary radium treatment Special attention has been paid to the lymph nodes along the iliac vessels which are recognized as highly likely to be involved by carcinoma even in the early clinical stages The topography of these nodes has also been correlated to the tissue volume irradiated homogeneously by supplementary external treatment

Methods Ten patients 5 with stage I b carcinoma and 5 with stage II a were chosen four of the patients had an exophytic tumor growth three had disc shaped tumors and in three patients the carcinoma was endocervical

Lymphography was performed after insertion of a cannula into a lymphatic vessel on the dorsal surface of the foot (RUTTMANN 1962 TJERNBERG 1962) Lipiodol Ultra Fluide (0.48 mg iodine/ml) was injected at a rate of 0.12 to 0.15 ml/min in a total amount of 8 ml to each side The lymph vessels running along the external and common iliac vessels may be observed during the injection procedures on films taken at this time The location and the characteristics of the lymph nodes may be studied twenty four hours later The external iliac lymph nodes and the common iliac lymph nodes may be subdivided into lateral intermediate and medial groups as already proposed The internal iliac lymph nodes are seldom demonstrated by this technique The subdivisions of the parietal nodes of the internal iliac lymph node system are the superior and inferior gluteal the obturator and the lateral sacral lymph nodes The visceral nodes of the same system are those of the bladder the para uterine tissues and the rectum (KUBIK et coll 1967) In the literature the lymph nodes between the external and the internal iliac arteries are sometimes called the interiliac nodes and are subdivided into the hypogastric nodes in the angle between the external and the internal iliac arteries and the obturator nodes in the obturator fossa cranial to the obturator artery (REIFENSTUHL 1967)

Phlebography was carried out after inserting polythene catheters percutaneously into both common iliac veins One hundred milliliters Urografin 45 % were injected into both tubes at a rate of 5 to 8 ml/sec when the tips of the catheters lay close to the internal iliac veins The inferior vena cava was blocked with a rubber balloon pressed against the abdomen with a plastic plate

Table 1

Corrected survival rate figures for those stage II b and III cases from the consecutive series of carcinoma of the uterine cervix, which were treated with conventional roentgen rays and ^{60}Co two opposed beams and ^{60}Co three beam technique respectively. Frequency of complications from bladder and rectum are given as 3 and 5 year corrected rates for the combined stage II b and III cases using the grading system defined by KOTTMEIER (1964). Figures obtained from an analysis of case reports at Radiumhemmet (JOELSSON 1970)

	Conventional roentgen rays four portals 1958—1959		Cobalt 60 two opposed beams 1960—1962		Cobalt 60 three beam technique 1964—June 1966	
	Stage II b	Stage III	Stage II b	Stage III	Stage II b	Stage III
Number of patients	138	57	42	65	37	71
Percentage of total	72 °	42 °	16 °	42 °	22 °	58 °
3 year corrected survival rate	61 °	34 °	53 °	25 °	52 °	43 °
5 year corrected survival rate	48 °	31 °	42 °	24	—	—
Bladder complications						
grade II to III						
3 year corrected rate	6 °		15 °		2	
5 year corrected rate	8 °		15 °		—	
Rectal complications						
grade II to IV						
3 year corrected rate	8 °		20 °		9 °	
5 year corrected rate	10 °		20		—	

During 1960—1962 some patients were treated with two opposed cobalt 60 beams, in 1964 a cobalt 60 three beam technique with individual dose planning was introduced.

The patients in the second and third groups were selected in that those with conditions more advanced than the average were treated with high energy radiation while those in the first group formed an unselected series. Within the respective clinical stages, the 5 and 3 year survival rates are not strikingly different in the three periods (Table 1). The only tendency towards the superiority of high energy therapy with doses of about 4 500 rad to the parametrium over 5 to 7 weeks, is found in the series of stage III patients receiving individually planned cobalt 60 by the three beam technique. However, the series are small and only 3 year follow up figures are available.

Several points must be considered as to the factors that may be used by the clinician as a guide in attempts to increase the cure rate. Interest at Radiumhemmet has for example been focused upon the correlation between the histology and prognosis (WETTERDAL 1934) as well as upon the possible prognostic guid-

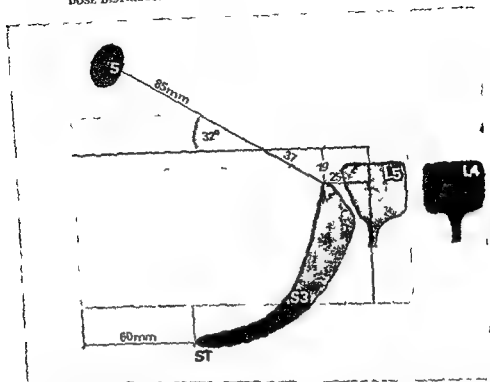


Fig 1 The relationship between the target volume and the pelvic structures. The ventral and cranial surfaces of the volume together with the limits of variation. Figures of distances are mean values in mm for the 10 patients under investigation. Due to individual differences in the inclination of the pelvis in supine position the angle formed by the ventral surface of the target volume and the line between the symphysis pubis (S) and the promontory of the sacrum (P) varied between 23° and 53° mean 37°. The mean distance between the caudal surface of the target volume and the tip of the sacrum (ST) was 60 mm. S3 = middle part of the third vertebra.

Supplementary external irradiation Dose planning was performed for external cobalt 60 therapy by the three beam technique developed at Radiumhemmet (RANUDD 1965). The target volume which was to be surrounded by the 95% isodose curve was carefully determined in each individual instance after thorough clinical examination of the anesthetized patient. The extension of the target volume in the ventrodorsal direction was defined by the size of the tumor as estimated bimanually with the addition of 2 cm ventrally and 2 cm dorsally. Laterally the target volume was extended to the pelvic wall and in the 10 patients was found to be 16 cm wide. The cranial-caudal range of the target volume was taken as 16 cm symmetrically distributed around the center of the tumor. This was represented in the a.p. and lateral roentgen films by means

held by a belt (HELANDER & LINDBOM 1955) The ap and lateral roentgen films were sometimes supplemented with oblique views

Thermoluminescence dosimetry Commercially available detectors, lithium fluoride (LiF) in teflon, 6 mm long and 1 mm in diameter, were introduced between spacers of lead, 7 mm long and 1 mm in diameter, in a teflon catheter, with an outer diameter of 1.8 mm Each catheter was loaded with 18 detectors

The teflon catheters were inserted percutaneously into the femoral veins immediately before the radium application under roentgen TV control until the tips lay in the inferior vena cava They were fixed in position and their location recorded with ap and lateral roentgen films The catheters remained in the veins during the primary radiation treatment of the patient which consisted of combined intra uterine and intravaginal radium application with the current Stockholm technique (KOTTMEIER 1964)

After removal of the radium from the patient the catheters were withdrawn and the separate detectors were recovered in order and numbered A partly rebuilt commercial instrument (Con Rad read out instrument, model 5100 A) was used for the determination of the dose on each detector, employing its individual calibration factor Various sources of error can be discriminated and reduced in using thermoluminescence LiF (CARLSSON 1969) The standard deviation varied between 6.6 and 8.7 % of the mean in a study of 10 detectors, treated in the same way as those in the study, and run through eight exposure events

Dose rate measurement in the bladder and rectum was performed with a Siemens Gammameter by a technique described in detail in a previous paper (JOELSSON & BACKSTROM 1969) The important features will be repeated

The centimeter graduated probe of the Siemens Gammameter was introduced into the urinary bladder and starting 12 to 13 cm cranial to the orifice of the urethra the values of dose rates were noted during its withdrawal Attention was paid to the mean of the three highest consecutive measurement values at centimeter intervals Similar measurements in relation to the anal sphincter were performed in the rectum

All the measurements were made with the patient supine immediately after the application of the irradiators They were repeated at the end of the treatment immediately before removal of the radium

The mean of the values determined at the beginning and at the end of the treatment were used as the definitive The fact that the Siemens Gammameter was calibrated at room temperature but used at body temperature was considered only when dose rates in bladder and rectum were related to doses measured by LiF dosimetry The observation that the reading of the instrument was lowered by 0.7 % per degree centigrade in temperature was made recently (JOELSSON & BACKSTROM 1969) Uncorrected figures have been used earlier and have also been correlated to frequency of complications (KOTTMEIER 1964)

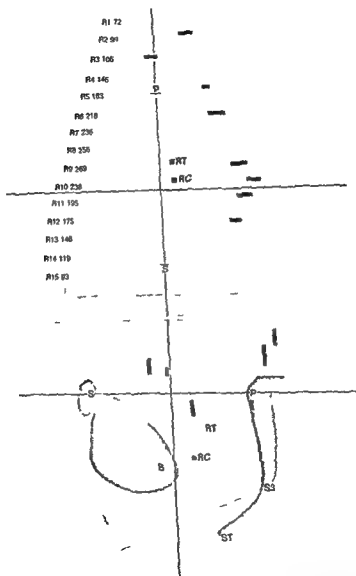


Fig 3 Stage I B Endocervical carcinoma 61 mg Ra 40 mm active length in uterus Flat box 71 mg Ra in vagina Treatment time 25 hours Bladder dose 110 rad rectal dose 1730 rad

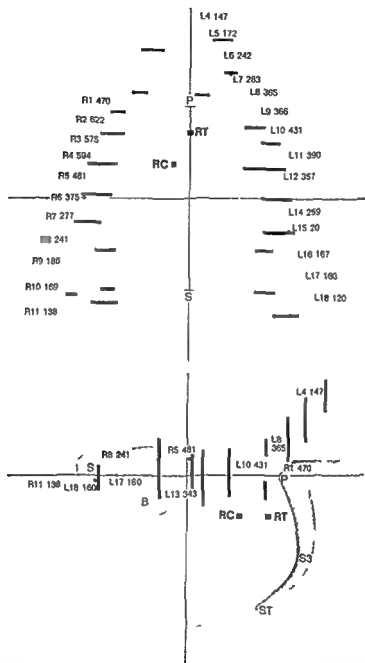


Fig 2 Stage I b Exophytic carcinoma. The intra uterine irradiator contains 61 mg Ra (active length 40 mm) and the vaginal flat box 71 mg Ra. Treatment time 24 hours. Dose at the posterior wall of the bladder 2 030 rad at the anterior wall of the rectum 2 260 rad.

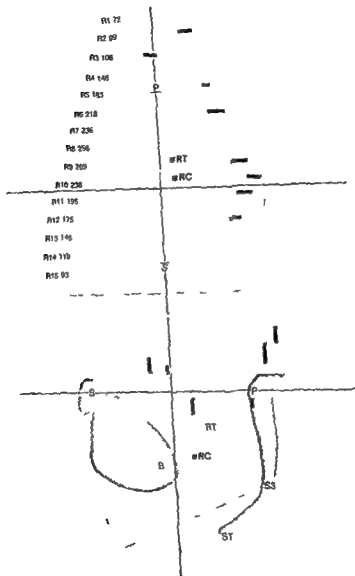


Fig 3 Stage I b Endocervical carcinoma 61 mg Ra 40 mm active length in uterus Flat box 71 mg Ra in vagina. Treatment time 95 hours. Bladder dose 2110 rad rectal dose 1730 rad

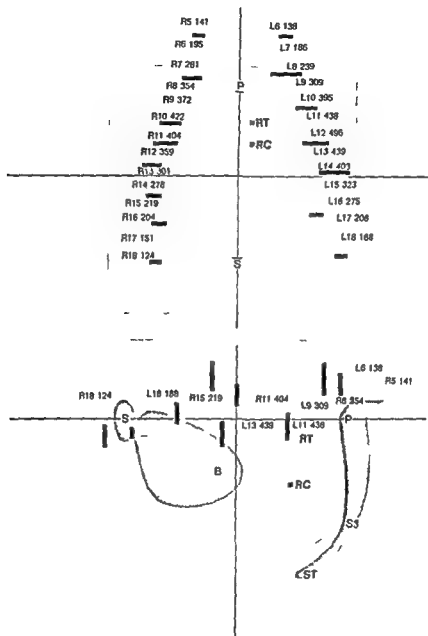


Fig 4 Stage I b Extraphytic carcinoma 43 + 54 mg R1 intra uterine tandem 16 + 27 mm active length 71 mg Ra in flat box in vagina Treatment time 25 hours 2 090 rad to the bladder 1 790 rad to the rectum

DOSE DISTRIBUTION IN CARCINOMA OF THE UTERINE CERVIX

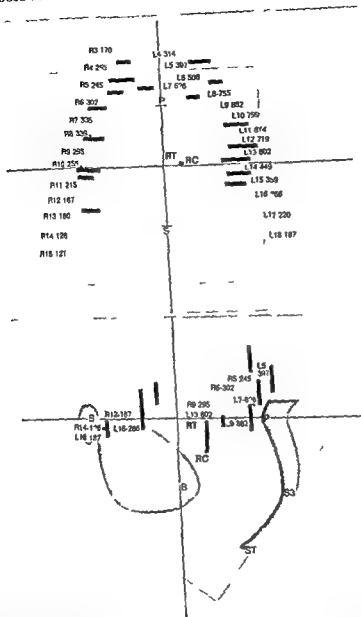


Fig. 5 Stage I b Endo cervical carcinoma 68 mg Ra 45 mm active length in uterus Flat box 71 mg Ra in vagina Treatment time 28 hours 2 700 rad to the bladder and 1 640 rad to the rectum

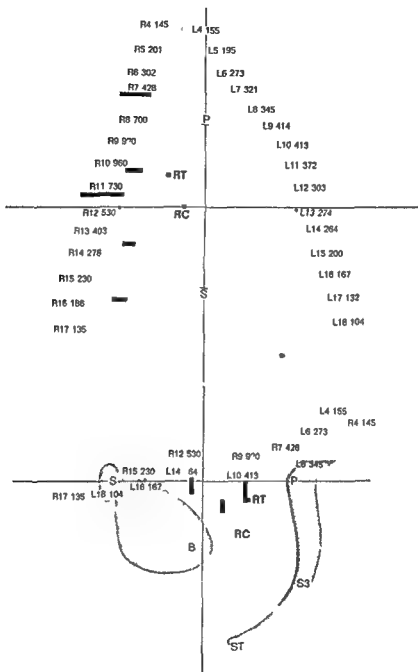


Fig 6 Stage I b Endocervical carcinoma 68 mg Ra active length 45 mm in uterus 71 mg Ra in flat box in vagina Treatment time 22 hours 2 410 rad to the bladder and 2 180 rad to the rectum

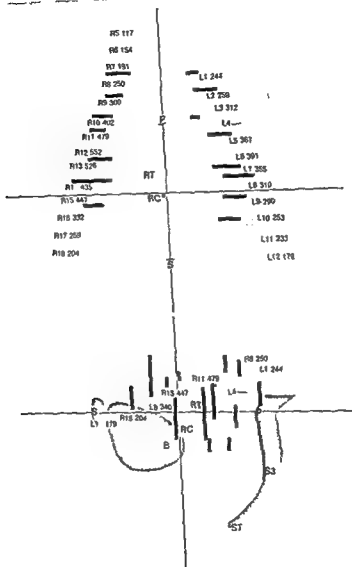


Fig 7 Stage II a Exophytic carcinoma cauliflower type 67 mg Ra active length 39 mm in uterus 80 mg Ra flat box in vagina Treatment time 22 hours 2380 rad to the bladder and 2640 rad to the rectum

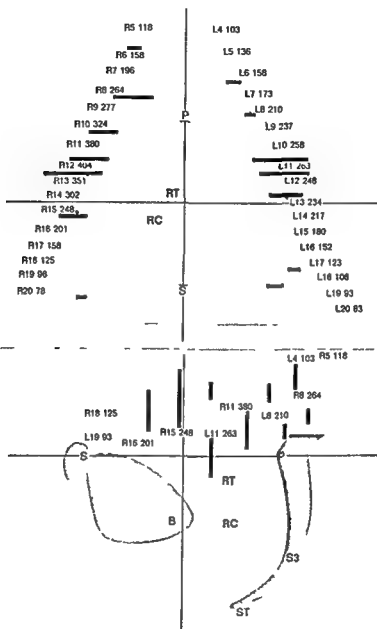


Fig II Stage II a Disc shaped carcinoma 70 mg Ra active length 54 mm in uterus 71 mg Ra in flat box in vagina Treatment time 26 hours 1 330 rrd to the bladder and 1 940 rad to the rectum

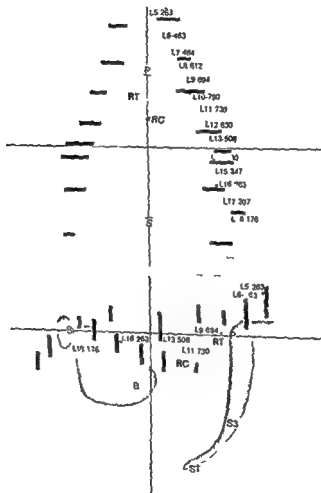


Fig 9 Stage II a Exophytic carcinoma 68 mg Ra active length 45 mm in uterus Flat box
mg Ra in vagina Treatment time 77 hours 2 980 rad to the bladder and 2 590 rad to
the rectum

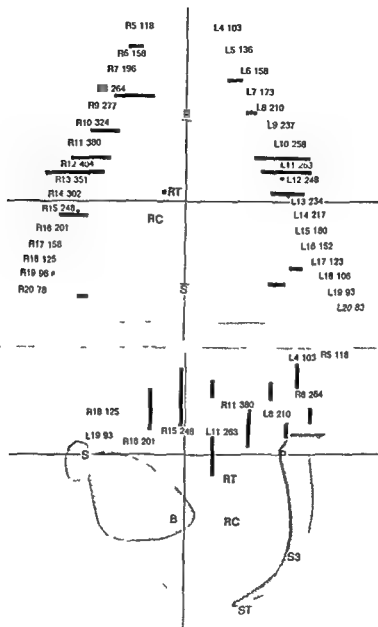


Fig 8 Stage II a Disc shaped carcinoma 70 mg Ra active length 54 mm in uterus 71 mg Ra in flat box in vagina Treatment time 26 hours 1 330 rad to the bladder and 1 940 rad to the rectum

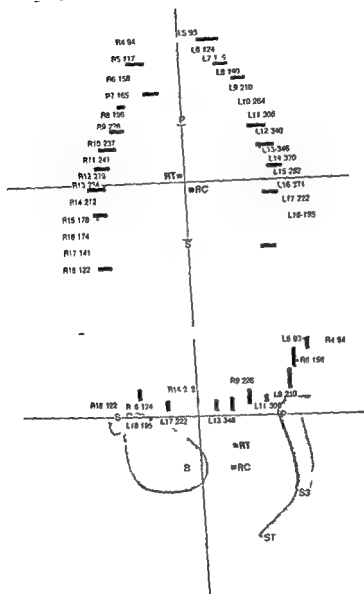


Fig 11 Stage II a II sc shaped carcinoma 62 mg Ra active length 39 mm in uterus 84 mg Ra in flat box in vagina Treatment time 96 hours 1430 rad to the bladder and 2020 rad to the rectum

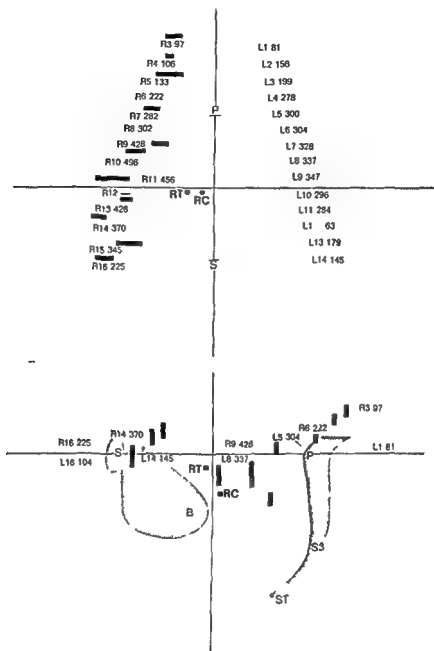


Fig 10 Stage II a Exophytic carcinoma 68 mg Ra active length 5.5 mm in uterus Curved box 77 mg Ra in vagina Treatment time 22 hours 1 800 rad to the bladder and 3 090 rad to the rectum

Table 2

Radiation doses during one course of intracavitary radium treatment to separate parts of the veins in rad as mean values and limits of determinations in ten patients. The values for the cranial and caudal parts of the common and external iliac veins and for the femoral vein are based on measurements of two LiF detectors for each patient. Correction has been made for the contribution from diagnostic roentgen procedures. Data on irradiators used are given in Figs 2 to 11.

	Inferior vena cava	Common iliac vein			External iliac vein			Under inguinal ligament
		Cranial A	Middle B	Caudal B	Cranial C	Middle D	Caudal D	
Right side								
Mean	110	173	195	300	410	473	381	310
Limits	53-171	87-300	116-273	175-468	230-808	259-958	252-628	210-445
Left side								
Mean	146	232	297	381	443	384	360	283
Limits	57-261	97-472	188-508	145-817	190-805	246-567	254-659	203-497

The location of the catheters in some of the patients allowed determinations to be made on detectors in the inferior vena cava and in the middle parts of the common and external iliac veins. The detector exactly posterior to the inguinal ligament was always considered separately. The complete list of determinations is given in Table 2.

Diagnostic roentgen films were taken with the LiF detectors in place. A special study disclosed that the contribution to the thermoluminescent signal from these procedures was 2 to 3 rad. The values in the text and in Table 1 are accordingly corrected while in Figs 2 to 11 the uncorrected dose determinations are given for each specific detector.

The effect on the thermoluminescent detectors from the lead spacers between them in the teflon catheters was investigated in a separate study although no measurable contribution to the signal was anticipated. The results of the measurements indicate no detectable influence on the signal of the detectors from the spacing with lead.

Topography of target volume for supplementary external irradiation. It was a constant finding that in the a.p. projection the cranial surface of the target volume was located 1 to 4 cm cranial to the promontory (Figs 2 to 11 upper halves). Related to the finding at phlebography the cranial surface of the volume crossed the middle parts of the common iliac veins. The frontal projection of the caudal surface of the target volume in all cases extended caudally to the symphysis pubis by 3 to 5 cm, thus in this view the caudal surface crossed the femoral veins.

of an opaque cylinder inserted into the cervical canal. Accurate body contours were drawn in each instance and the target volume in the transverse section recorded according to the above principles.

All the relevant information was transferred from the various roentgen films to one diagram with correction for distortion due to magnification. A line between that part of the symphysis pubis (S) projecting most posteriorly and the promontory of the sacrum (P) was chosen as the main axis of the system of coordinates in the frontal and lateral views. The representation of the lymph nodes, the projection of the centers of the lithium fluoride detectors, (R = right side, L = left side) and the projection of the center (RC) and the top (RT) of the intrauterine irradiator were all referred to this axis (Figs 2 to 11).

The surfaces circumscribing the target volume were indicated as their projection appeared in the frontal and lateral views. Metal frames were taped to the skin of the patient and demonstrated on roentgen film by the orthographic technique. The posterior wall of the urinary bladder (B) was indicated in the lateral view after the instillation of 50 ml Urografin 30 %.

Results

Dose rate measurements in the bladder and rectum The dose over one course of radium treatment on the posterior wall of the urinary bladder varied between 1 460 rad and 2 650 rad (mean 2 200 rad). The dose to the anterior wall of the rectum amounted to between 1 800 rad and 3 400 rad (mean 2 400 rad).

Thermoluminescence dosimetry in the pelvic veins The radiation dose to the common and external iliac veins were referred to four divisions (A—D) of the vessels, each 20 mm in length, corresponding to two LiF detectors and one interposed spacer. The right and left sides of the pelvis were considered separately because of the anatomic inequalities. A. In the cranial part of the common iliac vein, the dose varied between 87 and 300 rad on the right side and between 95 and 472 rad on the left side, mean values 173 rad and 232 rad respectively. B. In the caudal portion of the common iliac vein the dose on the right side was 175 to 468 rad, mean 300 rad and on the left side 145 to 817 rad, mean 381 rad. C. In the cranial part of the external iliac veins, distal to the confluence between the internal and external iliac veins the dose on the right side was 230 to 808 rad, mean 410 rad, while on the left side corresponding values were 190 to 805 rad with a mean of 443 rad. D. In the caudal part of the external iliac vein, cranial to the inguinal ligament the dose on the right side ranged between 252 and 628 rad, mean 382 rad, and on the left side between 254 and 659 rad, mean 360 rad.

to the promontory. The cranial surface of the target volume was evident at a distance of 0.7 to 4.5 cm (mean 2.5 cm) cranial to the promontory. The projection of the dorsal surface of the target volume, which was always parallel to the firm support of the patient, usually crossed the sacrum at about the middle of its third vertebra. The caudal surface of the tumor volume was located 2.5 to 8.0 cm (mean 6 cm) caudal to the tip of the sacrum.

It is apparent from the results that the projection of the ventral surface of the target volume crossed the distal part of the common iliac vein or the proximal part of the external iliac vein. The lymph nodes along the external iliac vessels were located outside the target volume in the ventral direction. The target volume enclosed the posterior part of the urinary bladder in all but one patient. Although the rectum was not demonstrated on the roentgen films it was concluded that a part was within the target volume.

Discussion

The fact that survival rates in carcinoma of the cervix uteri are about the same irrespective of differences in techniques should not produce a nihilistic feeling with no attempt being made to improve the results with the facilities now available. The survival rates in early stages of carcinoma of the cervix have already been raised to such a level that a reduction of adverse side effects of treatment may be considered a worthwhile objective on its own.

High energy therapy makes it possible to deliver a cancericidal dose to the whole pelvis with one or two ventral and dorsal beams, and at Radiumhemmet this technique has been applied in several patients with stage II b and III carcinoma of the uterine cervix. No individual dose planning was performed during the period 1960—1963. Between 2 000 and 5 000 rad (mean 3 700 rad) were delivered over 4 to 5 weeks. An analysis of the patients in stage II b who received cobalt 60 irradiation with two opposed beams in addition to one or two intracavitary applications of radium reveals that the 3- and 5-year survival rates were not statistically different from the figures for those who received conventional roentgen ray irradiation. The same applies to the patients with stage III carcinoma. The differences may be partly explained by the fact that only a small proportion of the total number of patients in stage II b during the time under review were treated with two opposed cobalt beams. In contrast to that in the roentgen ray series almost three fourths of the patients received the specified treatment, which invalidates too close a comparison between survival rate figures (Table 1). It is of interest however to find that the frequency of complications from the urinary bladder grade II to III was raised from 6% (conventional roentgen rays) to 15% (^{60}Co two opposed beams) and the

Table 3

Frequency of lymph node involvement (%) in stage I and II carcinoma of the uterine cervix. The patients underwent operation as primary treatment and lymphadenectomy was performed compulsory (From REIFENSTUHL 1967 and the references therein)

	Number of patients	Stage	
		I	II
ANTOINE (1959) (TROEWS & ULM)	544	8.5	27.9
DE BIASI (1954) (PAPADIS)	135	20.7	31.9
BRUNSCHEWIG & ROESLER (1957)	74	14.0	34.0
CARTER et coll (1953)	79	16.7	25.9
CATTANEO (1954) (MARZIALE)	66	20.0	—
CHRISTENSEN et coll (1955)	100	21.3	41.0
CURRIC (1962)	339	16.0	38.0
GRAY (1958)	61	11.0	35.0
GUSBERG et coll (1953)	64	10.0	31.0
KINDROUCH (1959)	84	34.0	51.0
LANCE (1960)	178	28.8	43.8
LIU & MEIGS (1955)	258	17.0	40.0
MARTINEZ et coll (1953)	442	23.0	41.0
MEDINA et coll (1959)	60	20.0	40.0
MEIGS & MORTON (1958)	130	18.0	37.0
MITANI et coll (1957)	182	39.9	33.6
MITANI et coll (1962)	78	30.2	—
MITRA (1959)	192	18.0	29.0
MORTON et coll (1952)	89	15.0	24.0
NAV RATIL (1955)	180	20.0	29.0
PARSON (1962)	80	13.0	22.0
SHERMAN et coll (1952)	176	12.0	29.0
TACHIBANA (1956)	416	12.0	23.7
WELCH et coll (1961)	383	12.0	24.0

below the inguinal ligaments. The lateral surfaces of the target volume in every instance enclosed the frontal projection of the lymph nodes although the margin was small.

In the lateral view the intersection between the projection of the ventral surface of the target volume and the line connecting the promontory of the sacrum with the symphysis pubis was located a sixth to a half (mean a third) of its length from the promontory (Fig. 1 and Figs 2 to 11, lower halves). The angle between the projection of the ventral surface of the target volume and the conjugate diameter varied between 23° and 53° (mean 32°). The lateral projection of the ventral surface of the target volume was 0.8 to 3.7 cm (mean 1.9 cm). Ventral

tissue. It is reasonable to postulate that a modification of the technique of external irradiation to include the lymph nodes along the external iliac vessels in the target volume would be to the advantage of the patient and lead to an improvement in the survival rates. Changes have therefore been considered along different lines. The simplest modification implies an increase in the load on the ventral beam by a factor of two or more. If high energy electrons are available another choice will be two frontal electron beams as a supplement to the original three beam technique. The electron portals can be designed so that they obliquely transverse the frontal cobalt 60 field and cover the lymph node chain only. The energy of the electrons must be adapted to the individual patient within the limits of 15 to 25 MeV.

SUMMARY

A series of patients examined with lymphography, phlebography, LiF dosimetry and localization of the target volume disclosed that the pelvic wall dose from intracavitary treatment was small and that the three beam technique for external cobalt irradiation failed to include the lymph nodes along the external iliac vessels. This finding necessitates a modification of the technique. A redistribution of the loads of the three beams or the addition of high energy electron beams are suggested.

ZUSAMMENFASSUNG

Es zeigte sich bei der Untersuchung von einer Serie von Patienten mittels Lymphographie, Phlebographie, LiF Dosimetrie und nach Messung der Tiefendose, dass die Dosis zur Wand der Pelvis bei intrakavitärer Behandlung niedrig war und dass die Dreifelderbestrahlung mit der Kobaltbombe die Lymphknotengruppe entlang der Iliaca externa nicht genügend erfasst. Hieraus ergibt sich, dass eine Änderung der Technik nötig ist. Eine Neuverteilung der Felder ist notwendig oder die Zusage von zusätzlichen Feldern von hochgeschwindigen Elektronenstrahlen.

RÉSUMÉ

Les examens au moyen de lymphographie, phlebographie, dosimétrie par LiF et la localisation du volume cible chez une série de malades atteintes de cancer du col de l'utérus ont montré que la dose à la paroi du bassin est petite et que la technique d'irradiation externe au cobalt par trois champs n'inclue pas les ganglions lymphatiques situés le long des vaisseaux iliaques externes. Cette constatation impose une modification de la technique. Les auteurs proposent une répartition différente des doses faites par les trois champs ou l'addition d'une irradiation par des électrons d'haute énergie.

REFERENCES

- MIREN, C. A. und TCHOKE, S. Lymphknotenbefunde nach Wertheim Meigscher Operation. *Geburtsh. u. Frauenheilk.* 21 (1961) 219.
CARLSSON, C. A. Thermoluminescence of LiF: dependence of thermal history. *Phys. in Med. Biol.* 14 (1969) 107.

frequency of rectal complications grade II to IV from 8 % to 20 % (For the grading system the reader is referred to KOTTMER 1964)

It was hoped that a more accurate administration of the dose to the tumor area and especially to the internal iliac and common iliac lymph nodes with the three beam technique established in recent years might improve the results without causing damage to normal tissue. This technique in combination with individual dose planning and absorbers of special size and shape in the frontal beam during a number of the fractions also reduces the dose to the anterior part of the bladder and posterior part of the rectum. Only three year figures are available at the present time and even if these do not consistently indicate any superiority of the technique as regards survival, a clear tendency towards a decreased incidence of complications exists in spite of an average dose of 4500 rad (2500 to 6700 rad) over 5 to 7 weeks.

The routes of spread of carcinoma of the cervix are into the vaginal mucosa, into the myometrium of the lower uterine segment and into the network of lymphatics of the paravaginal as well as the parametrial tissues. Combined statistics in the literature present figures of involvement of the nodes at the pelvic wall of between 15 and 20 % in stage I carcinoma, and 30 to 35 % in stage II carcinoma (Table 3). The variation in the figures are to some extent dependent upon whether single or serial sections of the lymph nodes have been studied. A considerable increase in lymph node involvement has been observed by some authors when serial sections have been examined (LANGE 1960, AHRENS & TSCHOKE 1961, HUIHN 1962 and MITANI et coll 1962). It is known that a considerable proportion of involved nodes are to be found along the external iliac vessels (cf REIFFENSTUHL 1967). The doses to the common and the external iliac vein are comparatively low with intracavitary radiation methods and compared to the doses to the posterior wall of the bladder and the anterior wall of the rectum the doses at the pelvic wall are five to eight times smaller. Doses of similar magnitude measured both by intravascular thermoluminescence detectors (TJERNBERG et coll 1968) and with an ionization chamber during the procedure of extraperitoneal lymphadenectomy (KOTTMER 1951) have been reported earlier. The correlation between isodose rates around the combined intra uterine and vaginal irradiators and pelvic anatomy reported by WALSTAM (1954) are also in agreement with the observations in the present study.

The magnitude of the supplementary dose to be given to the parametrium by external irradiation is determined in each instance by the stage of the disease and condition of the patient. The three beam technique produces steep dose rate gradients towards the surrounding tissue, and necessitates the inclusion of relevant tissues in the target volume. The observation is described in this paper that the routine three beam technique may well exclude some potentially malignant

tissue. It is reasonable to postulate that a modification of the technique of external irradiation to include the lymph nodes along the external iliac vessels in the target volume would be to the advantage of the patient and lead to an improvement in the survival rates. Changes have therefore been considered along different lines. The simplest modification implies an increase in the load on the ventral beam by a factor of two or more. If high energy electrons are available another choice will be two frontal electron beams as a supplement to the original three beam technique. The electron portals can be designed so that they obliquely transverse the frontal cobalt 60 field and cover the lymph node chain only. The energy of the electrons must be adapted to the individual patient within the limits of 15 to 25 MeV.

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REFERENCES

- ANDREAS C. A. und TSCHOKE S. Lymphknotenbefunde nach Wertheim Meigscher Operation. *Geburtsh. u. Frauenheilk.* 21 (1961) 219.
 CARLSSON C. A. Thermoluminescence of LiF: dependence of thermal history. *Phys. in Med. Biol.* 14 (1963) 107.

- HELANDER C G and LINDBOM A Sacrolumbar venography Acta radiol 44 (1955) 410
- HUHN F O Drüseneinschlüsse in Beckenlymphknoten und Endometriose Geburtsh u Frauenheilk 22 (1962) 335
- JOELSSON I A study of three different techniques of supplementary external irradiation in carcinoma of the uterine cervix with special reference to frequency of complications and to survival rate To be published in Acta radiol
- and BACKSTROM A Dose rate measurements in bladder and rectum Intracavitary radiation of carcinoma of the uterine cervix Acta radiol Ther Phys Biol 8 (1969) 343
- KJELLGREN O The radiation reaction in the vaginal smear and its prognostic significance Studies on radiologically treated cases of cancer of the uterine cervix Acta radiol (1958) Suppl No 168
- KOTTMEIER H L Studies of the dosage distribution in the pelvis in radium treatment of carcinoma of the uterine cervix according to the Stockholm method J Fac Radiol (Lond) 2 (1951), 312
- Do serial biopsies disturb the healing process of the irradiated cervical canal Acta cytol (Philad) 3 (1959) 397
- Surgical and radiation treatment of carcinoma of the uterine cervix Acta obstet gynec scand (1964) Suppl No 2
- Complications following radiation therapy in carcinoma of the cervix and their treatment Amer J Obstet Gynec 88 (1964) 854
- KUBIK S TONDURY G RÜTTIMANN A and WIRTH W Nomenclature of the lymph nodes of the retroperitoneum the pelvis and the lower extremity In Progress in lymphology p 52 Georg Thieme Verlag Stuttgart 1967
- LANGE P Clinical and histological studies on cervical carcinoma Precancerous early metastases and tubular structures in the lymph nodes Acta path microbiol scand (1960) Suppl No 143 p 9
- MITANI Y, FUJII J, MIYAMURA M et coll Lymph node metastases of carcinoma of uterine cervix Amer J Obstet Gynec 83 (1962) 515
- RANUDD N F Dose distribution studies in external irradiation of carcinoma colli uteri Acta radiol Ther Phys Biol 4 (1966) 353
- REIFENSTUHL G Das Lymphknotenproblem beim Carcinoma colli uteri und die Lymphirradiatio pelvis Urban & Schwarzenberg München 1967
- RUBIO C A, HERTZBERG O, KOTTMEIER H L et coll Sensitization and radiation response in cases with carcinoma of the uterine cervix Acta radiol Ther Phys Biol 3 (1965) 241
- RÜTTIMANN B Die Lymphographie mit öligen Kontrastmittel Fortschr Röntgenstr 97 (1962) 551
- SELDINGER S I Catheter replacement of the needle in percutaneous arteriography A new technique Acta radiol 39 (1953) 368
- TJERNBERG H Lymphography An animal study on the diagnosis of Vx2 carcinoma and inflammation Acta radiol (1962) Suppl No 214
- JOHANSSON J M och LINDSKOUG H Thermoluminescensdosimetri vid radiologisk behandling av gynekologisk cancer klinisk tillämpning (In Swedish) Nord Med 80 (1968) 1537
- WALSTAM R The dosage distribution in the pelvis in radium treatment of carcinoma of the cervix Acta radiol 42 (1954) 237
- WETTERDAL P Does the microscopical diagnosis afford prognostic guidance in cervical cancer? Acta obstet gynec scand 14 (1934) 302

WHOLE BODY MEASUREMENTS ON THE DISTRIBUTION OF MERCURY-203 IN HUMANS AFTER ORAL INTAKE OF METHYLRADIOMERCURY NITRATE

by

R. FALK, J. O. SÄMS, L. ERMAN, U. GREITZ and H. ÅBERG

The presence of mercury contaminants in nature has been proven in the past few years mainly in fresh water fish. Since as much as 12 mg/kg (wet weight) of mercury has been reported in Sweden, it must be assumed that the lower consumption of fish is the reason for the avoidance of such mercury poisoning as have occurred in Minamata and Mugatu in Japan. Sale of fish containing more than 1 mg of mercury per kg wet weight is now forbidden in Sweden.

Irrespective of the chemical form of the mercury contained in the effluents methyl mercury is the ultimate product form after the process of methylation occurring in nature. In order to study in more detail the uptake and metabolism of methyl mercury in the human, three male volunteers, 37, 42 and 44 years of age, each took 2.6 μ Ci of monomethyl 203 mercury nitrate orally. Measurements were made for a period of more than two months on urine and faeces, and for more than seven months on total body activity. The result of the whole study has been presented by ÅBERG et coll. (1969). The purpose of the present report is

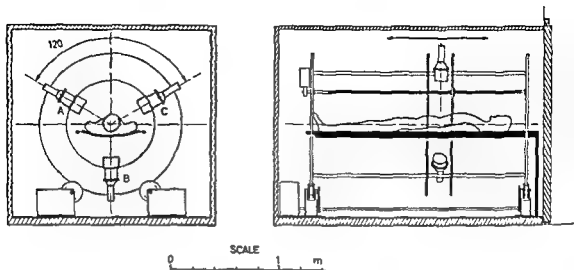


Fig 1 The three crystal counter

to give a detailed description of the distribution measurements with the whole body counter employed. Results of the distribution measurements are given, as well as a description of the methods used for measurement, calibration and sources of error. The report should thus give a picture of the possibilities provided,

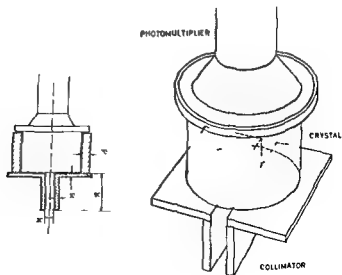


Fig 2 The collimator arrangement

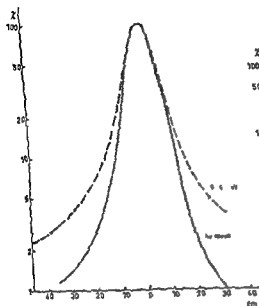


Fig 3 Profile curves obtained from a ^{203}Hg source with different energy intervals

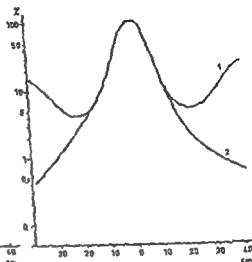


Fig 4 Comparison of the resolution with the present (2) and the previous (1) collimator arrangement

as well as the limitations of the whole body counter technique for similar investigations

Counters and collimators The measurements were carried out with the three crystal counting apparatus in the low background laboratory of the National Institute of Radiation Protection Stockholm. The apparatus has been described in detail previously (LIVDELL & MAGI 1966; MAGI 1967). The human subjects to be measured were in a supine position with the crystals placed symmetrically around them. For each measurement the crystals were moved axially with respect to the length of the body at least 2 cm (step scanning) (Fig 1). Each crystal 5×4 NaI(Tl) was provided with a simple slit collimator of lead having a thickness of 10 mm and a height of 90 mm (Fig 2). Each measurement was run for at least 3 minutes. The crystals were connected to a pulse height analyzer with a magnetic tape recording device.

For the drawing of the profile curve the net counts per minute in channels 22–31 corresponding to the energy interval 220–320 keV were used (^{203}Hg decays to 82% via 279 keV γ radiation). In this way the resolution of the profile

curve was better than if the entire energy interval, 0—4 000 keV, had been used (Fig. 3). This improvement in the resolution did not adversely affect the statistics.

During the period of the investigation (14 weeks), the collimators were in the process of improvement. The resolution for the two collimations used appears in Fig. 4, the less good of the two was used in the initial phase of the series of measurements (EKMAN, GREITZ, MACI *et coll.* 1968) but was found to give an insufficient resolution for the results sought. This part of the measurements has therefore not been taken into account in the presentation and discussion of the results.

Background measurements. No opportunity was provided for making whole-body measurements of the subjects prior to the intake of the ^{203}Hg . To provide the necessary background count, a 4th subject whose weight and length correspond to the average of the three subjects, was measured in the whole body counter. A summary of the gross counts (subject plus apparatus background) within the energy interval used for 12 subjects registering activity only from ^{137}Cs (from fall out) and natural activity (mainly ^{40}K), is given below.

Subject	Gross counts in cpm
1	448
2	406
3	423
4	406
5 (subject used as background)	465
6	405
7	419
8	395
9	428
10	410
11	406
12	471
	424 ± 24

These measurements were made by scanning the length of the body without collimation, and therefore give an indication of the distribution of the background among the 12 subjects. The activity of the subject used as background is marked in the table, showing that his activity was higher than the average (though within $\pm 2\sigma$). The result of the measurement of the profile for this subject is shown in Fig. 5. Collimators were used for this measurement. The background used is the average of the profile, which implies that the net values measured on

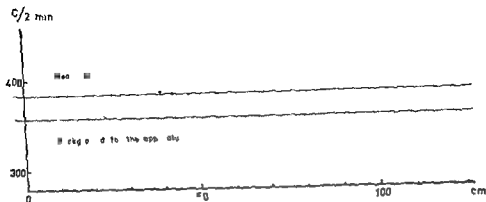


Fig 5 Profile curve for a background subject

the subjects are somewhat low in the area of the head and high in the trunk region on the assumption that the background used is the true background. This consideration has a certain importance for the accuracy of the final measurements that are discussed below.

Definition of the regions The aim of the measurements was to study the relative distribution and possible redistribution of the activity in the human body. For this purpose the profile curve was divided into five regions including the following organs: region (1) brain, (2) thyroid, (3) liver and spleen, (4) bladder and (5) thigh muscles.

As the division was made with respect to the anatomical details, the linear extension of the regions were not equal.

Region boundaries	A	B	C	D	E	F
Regions	1	2	3	4	5	

(A) skull cap (B) a plane below tragus at $1/4$ of the distance between tragus and jugular notch (C) jugular notch (D) umbilicus (E) ischial tuberosity and (F) knee

Identification of the organs As can be seen from the profile curve (see Fig 6) two marked peaks appear: one in region (1) and the other in region (3). Attempts were made in three different ways to identify the organs in these regions.

1 **Determination of the linear size of the organ** (parallel to the length of the body) corresponding to the peaks in the profile curve. Profile curves were taken of samples with various linear sizes to determine the FWHM (the full width of

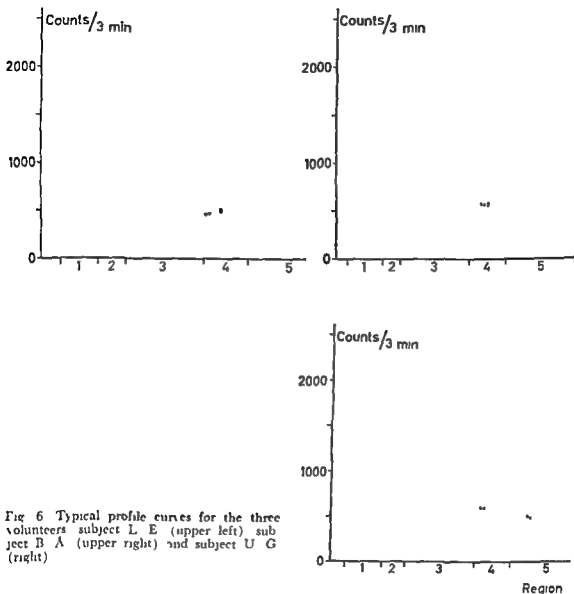


Fig. 6 Typical profile curves for the three volunteers: subject L. E. (upper left), subject B. A. (upper right) and subject U. G. (right).

half maximum) (see Fig. 7). Thus the size of the organ can be determined using the relationship $d_0 < \text{size of the organ} < d_1$ where $d_0 > 0$. For the peak in region (1) the size of the organ was determined to be < 15 cm and for the one in region (3) $10 \text{ cm} < \text{size of the organ} < 20 \text{ cm}$.

2. *Determination of the lateral position* was achieved by making measurements with one crystal at a time, the net counts indicating the asymmetrical position of the organ. The results of the measurements in region (3) using the experi-

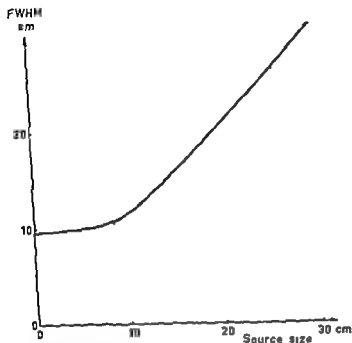


Fig 7 Full width of the half maximum of the profile curve as a function of source size

mental set up shown in Fig 1 were as follows with the counting rates in per cent

	Crystal A	Crystal B	Crystal C
Subject L E	23	37	40
Subject U G	20	37	43

The results indicate that the organ is situated on the right hand side of the body

3 *Determination of the longitudinal position* requires measurement of the profile. It can be seen from Fig 6 that the position of the organ in region (1) lies in a plane perpendicular to the length of the body and through the eyes and the occiput of the skull.

These three observations lead to the conclusions (A) that the major part of the activity in region (1) is concentrated within a small sub-region the position of which indicates that it may be the cerebellum and (B) that the major part of the activity in region (3) appears to be accumulated in the liver. In the other regions no specific site of the activity can be deduced.

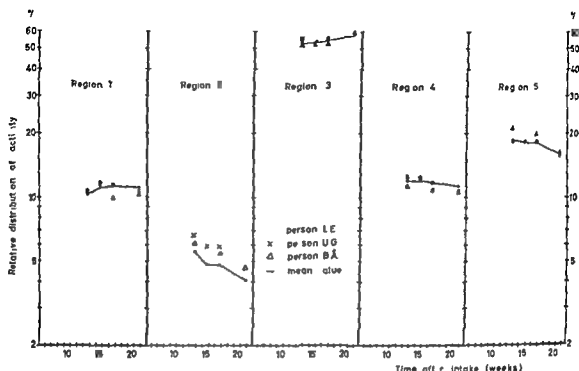


Fig. 8. Relative distribution of activity in different regions as a function of time. The figures are not corrected for systematic errors.

Distribution of activity, accumulation and biologic half lives

A profile curve of net counts within the peak was drawn on a linear scale for each subject and measurement. After marking the five regions, the areas under the curves were cut out and weighed. In this way the weight represented the total counts within each region. The results are shown in Fig. 8. The following conclusions may be drawn:

1. Deviations among the three subjects were small and it seems probable that they can be regarded as non significant when the errors (discussed below) have been taken into account. The average of the three subjects has been taken for each measurement and connected in the diagram by a line.

2. The lines indicate a gradual accumulation of the activity in regions (1) and (3), corresponding to the cerebellum and the liver. This is compensated for by a relative decrease in the other regions as can be seen from Fig. 8. The most rapid depletion appears to take place from region (2) (thyroid). The uncertainty in this region is, however, relatively large (see below).

The same result can be seen in Table 1 which summarizes the biologic half lives. Results have been included which have been calculated from the data

Table 1
Summary of the biologic half lives in days

Subject	Regions						
	Previous experimental set up	New experimental set up					
	1—3	1—5	1	2	3	4	5
L E	61	69	95	36	72	59	57
U G	75	70	95	48	68	73	68
B A	78	60	64	45	73	54	49
Mean	71	66	85	43	71	60	58

The half life for region (2) is probably somewhat underestimated because of the systematic errors discussed below

Table 2
Distribution in per cent of ^{203}Hg — The total activity in regions 1—5 is set to 100

Subject	Date	Regions				
		1	2	3	4	5
L E	26/3	9.7	4.1	54.0	13.0	19.2
	8/4	10.4	3.3	54.2	13.2	18.9
	22/4	10.4	3.1	55.2	12.2	19.1
	22/5	11.1	2.5	58.6	11.3	16.5
U G	27/3	8.8	6.7	54.4	13.1	17.0
	9/4	9.5	6.0	53.8	12.8	17.9
	23/4	11.0	6.0	52.4	13.5	17.1
	20/5	9.5	5.3	55.1	13.5	16.6
B A	25/3	9.3	6.3	50.5	11.9	22.0
	10/4	10.6	5.6	51.5	12.0	20.3
	24/4	8.9	5.6	53.5	11.3	20.7
	29.5	9.2	4.7	58.2	11.1	16.8

obtained in measurements using the previous experimental set up with the less satisfactory collimators. This fact does not impair the result for this case.

The half lives have been determined from diagrams in which counts corrected for radioactive decay are plotted versus time for each region (see Fig. 9). The biologic depletion has been assumed to occur at the rate represented by the

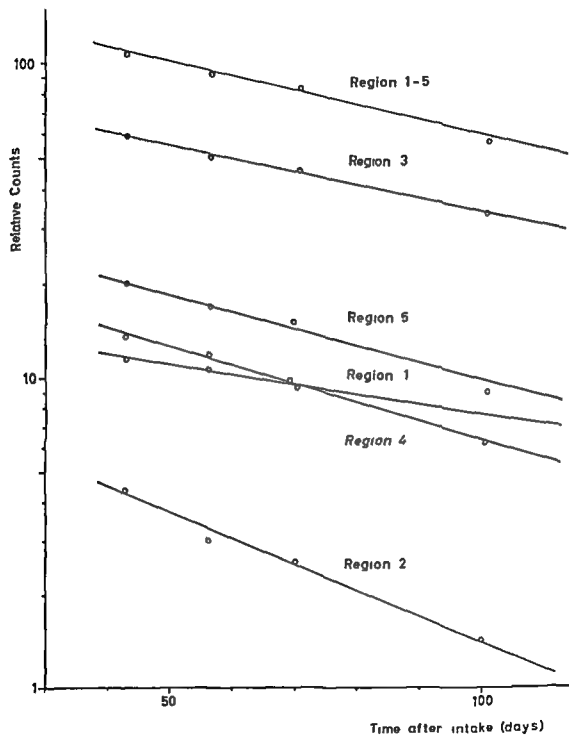


Fig 11 Determination of the biologic half life of methyl ^{203}Hg for subject I

Table 3
Distribution of ^{203}Hg in nCi

Subject	Date	Regions					
		1	2	3	4	5	1-5
L E	26/3	90	40	500	120	180	990
	8/4	70	20	350	90	120	650
	22/4	50	10	260	60	90	410
	22/5	20	5	120	20	30	210
U G	27/3	80	60	470	110	150	860
	9/4	60	40	350	80	120	640
	23/4	50	30	220	60	70	430
	20/5	20	10	120	30	40	220
B A	25/3	90	60	510	120	220	1000
	10/4	70	40	330	80	120	640
	24/4	40	30	240	50	90	450
	29/5	20	10	100	20	30	180

straight line drawn in the figure which corresponds to a one phase exponential process

To obtain an indication of the absolute distribution of the activity the number of counts must be corrected for absorption. The following assumptions have been made concerning the activity in the various regions, taking into account the previous identification of the organs (1) all activity in the cerebellum (2) activity evenly distributed (3) all activity in the liver (4) activity evenly distributed (5) activity evenly distributed

By making measurements with water phantoms the relationship between number of counts and activity was determined for the five regions. The activity was either evenly distributed throughout the phantom corresponding to regions 2) (4) and (5) or concentrated as in regions (1) and (3). The correction factor is expressed in nCi/mg from which after multiplication with the weight of the area under the profile curve, the activity is obtained in nCi. The correction factors for the five regions were for (1) 0.76 for (2) 0.86 for (3) 0.84 for (4) and (5) 0.88 nCi/mg. The distribution of the activity is shown in Table 2. In Table 3 the distribution of the activity is expressed in nCi.

Comparison between the measured and the expected total activity. As has been pointed out in the introduction the three subjects took an amount of ^{203}Hg

Table 4

The statistical counting error in subject L. E. at different measurement dates

Date	Region	Per cent	Date	Region	Per cent
26/3	1	2	22/5	1	5
	2	4		2	25
	3	1		3	2
	4	2		4	11
	5	3		5	8

orally on the 12 February 1968, which was measured to be $3.0 \mu\text{Ci}$ on the 2 February 1968.

Measurements on urine and faeces (EKMAN, GREITZ, PERSSON & ABERG 1968) on the days immediately following the intake, showed that the uptake was nearly 100 %. Based on the data of the distribution of the activity on the 25–27 March, and on the biologic half life which was found with the previous collimation, (see Table 1), the total activity was determined for the 2 February 1968. The result for subject L. E. was $3.6 \mu\text{Ci}$, for U. G. $3.1 \mu\text{Ci}$ and for B. A. $3.4 \mu\text{Ci}$.

The correspondence must be regarded as satisfactory when consideration is given to the simplicity of the phantom models used in the comparison measurements, and that the experiments a priori were not set up to be absolute measurements.

Sources of error and their influence on the results

I Counting statistics The statistical counting errors increased as the experiments progressed, since the decrease in the activity could not in practice be compensated for by correspondingly longer measurements. Table 4 shows the error in per cent in the five regions for the first and last measurements in subject L. E.

II Changes in position It was difficult to avoid slight movement of the subjects during the course of the relatively long measurements. Thus, longitudinal shifts of some centimetres could occur. Significant shifts in position were checked by a measurement of the distance between the top of the head and the top of the liver. Nevertheless movements in one or other direction involve an error of $\leq 4\%$.

III Background The fact that the correct background for each subject could not be used could have introduced an error which would be largest in the final

Table 5

Errors in per cent of the distribution percentage for the five regions at different measurement dates

Date	Reg on	Random error (%)	Systematic error (%)	Systematic error (%) when error VII=0
26/3	1	5	+16	+7
	2	5	-24	-30
	3	5	-6±2	-7±2
	4	5	-14±2	-10±2
	5	5	-11±2	-7±2
22/5	1	7	+25	+16
	2	5	+4	+8
	3	5	-6±7	-7±7
	4	9	-14±4	-10±4
	5	9	-11±8	-7±8

measurements in the series. To be able to obtain an estimate of the size of this error the activity distribution was calculated with three different background values (1) background constant over all the five regions (2) different background for each region corresponding to a background profile, and (3) different background for each region corresponding to the background profile for the subject having the lowest background (see tabulated data p 58). The conclusions were the following. The scatter of the three alternative values for the different regions was largest in the final measurement. The absolute differences between the highest and the lowest values were for region (1) $\approx 14\%$ (2) $\approx 0.6\%$ (3) $\approx 3.6\%$ (4) $\approx 0.5\%$ and for (5) $\approx 1.1\%$. Irrespective of which background value was used, the same tendency to increase or decrease is noted in the various regions.

IV Influence of adjacent regions Since the crystals see a larger field than that corresponding to the width of the slit a certain overlap of adjacent regions occurred. The most serious overlap occurs in region (2) from activity in region (3). Region (2) was therefore studied in subject L. E. who was the shortest of the three and in whose case the influence between the regions can be expected to be the greatest. Using a ^{203}Hg source of suitable size a profile which corresponded to the top of region (3) was measured. From this the influence on region (2) was calculated. The practical result was that approximately 10% of the number of counts measured in region (2) are due to activity actually in region (3). In the other regions the mutual influence was of little significance.

Table 4

The statistical counting error in subject L E at different measurement dates

Date	Region	Per cent	Date	Region	Per cent
26/3	1	2	22/3	1	5
	2	4		2	25
	3	1		3	2
	4	2		4	11
	5	3		5	8

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III Background The fact that the correct background for each subject could not be used could have introduced an error which would be largest in the final

estimate them. The error discussed under III is systematic in character but could not be estimated for each subject. The possible deviation was estimated by calculations the results of which are given under III. To determine the sign of the error for each subject, the ratio peak/total net counts was calculated for each region in the first and final measurements. This ratio should remain constant if the correct background had been used. However it is seen that the ratio peak/total counts is clearly larger for the final measurements than for the first for regions (1) and (2) thus giving the error a positive sign, i.e. the correct value is higher than the measured value. For the remaining regions this significant difference is not noted, thus giving either positive or negative errors. In contrast to the other systematic errors (see below) error III increases with time. Error V is obviously systematic and implies that region (1) was weighted too lightly, while the others were weighted too heavily. Finally, error VII is systematic. The percentage errors of the distribution per cent in the five regions for the first and the final measurements are summarized in Table 5.

It should be pointed out that no results in the text, the tables or the figures have been corrected for the systematic errors. If the total activity is corrected for the resulting maximum systematic errors a higher value for the calculated activity intake in μCi is found. As is seen above this value is already somewhat too high even without this correction. Concerning the biologic half lives (Fig. 9 and Table 1) and the changes in relative distribution of activity in the five regions (see Fig. 8) the systematic errors in IV, V and VII have no significant influence on the results. If a correction is made for all the systematic errors the percentage distribution of the activity in the five regions is changed as follows: an increase in region (1) of 1–3 % (absolute %) in region (3) of 0–2 % while the changes in the other regions are less. Relatively the systematic correction is large in region (2) at 25 % and in region (1) at 15 %–20 %. A major part of the systematic error originated from the use of an incorrect background. A further source of error which could be decreased was the collimation. The collimators used cut off a large part of the detector areas leading to impaired sensitivity. With focussing slit collimators both the sensitivity and the resolution could be improved.

Resulting radiation doses and ethical problems. The resulting doses of radiation were calculated for the following organs and on the basis of the points below:

- 1 Intake 3 μCi ^{203}Hg
- 2 100 % uptake
- 3 10 % in the cerebellum 54 % in the liver
36 % in the other parts of the body
- 4 Biologic half life 85 days for the cerebellum

V The division into regions Due to practical considerations, the profile scanning could not be carried out along the entire length of the body, and had to be limited to the area from the top of the head to the knee. Thus the regions beyond the skull cap and below the knee were not included. If these regions are extrapolated on the basis of the most feasible extensions of the measured profiles, an alternative percentage distribution will be found. For the region including the brain, which was studied from this point of view experimentally, an increase in the percentage of activity of only 0.5% was found (absolute %). With the boundary lines used, over 90% of the total body activity was covered, and approximately 90% of the activity in the region including the brain.

VI Cutting and weighing errors As mentioned above, the profile curves of the net counts within the ^{203}Hg total absorption peak were drawn on linear diagrams, cut out and weighed. The random errors introduced by this process were determined to be at a maximum 2%.

VII Absorption effects Possible absorption effects were studied by drawing profile curves from measurements on phantoms consisting of ^{203}Hg solutions of varying geometry and homogeneity. The result is expressed in nCi/mg (see above), or in mg/nCi, indicating the sensitivity. It could thus be seen that, using a phantom corresponding to the head (region 1), the sensitivity was increased by 13% when the activity was concentrated in the cerebellum, as compared to when it was homogeneously distributed. With phantoms corresponding to the trunk, the sensitivity was increased by 5% when the activity was localized at the position of the liver, as compared to a homogeneous distribution. For the calculation of the activity in the five regions in the subjects measured, we have, as mentioned above, assumed that the activity was concentrated in the cerebellum and the liver, in regions (1) and (3), respectively, and homogeneously distributed in the other regions. It may be that a systematic error was thus introduced due to the possibility that there may have been some activity in regions (1) and (3) not concentrated in the organs mentioned. The greatest error thus introduced can be calculated by assuming a homogeneous distribution. In this case the activity is increased by 13% in region (1) and by 5% in region (3). The percentage distribution of the activity is thus changed such that the percentage in region (1) is increased by 9%, in region (2) decreased by 4%, in region (3) increased by 10% and in regions (4) and (5) decreased by 4% (relative %).

Discussion

Comments on the resulting errors Of the errors discussed above those under

estimate them. The error discussed under III is systematic in character but could not be estimated for each subject. The possible deviation was estimated by calculations the results of which are given under III. To determine the sign of the error for each subject the ratio peak/total net counts was calculated for each region in the first and final measurements. This ratio should remain constant if the correct background had been used. However it is seen that the ratio peak/total counts is clearly larger for the final measurements than for the first for regions (1) and (2) thus giving the error a positive sign, i.e. the correct value is higher than the measured value. For the remaining regions this significant difference is not noted thus giving either positive or negative errors. In contrast to the other systematic errors (see below) error III increases with time. Error V is obviously systematic and implies that region (1) was weighted too lightly while the others were weighted too heavily. Finally error VII is systematic. The percentage errors of the distribution per cent in the five regions for the first and the final measurements are summarized in Table 5.

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- 1 Intake 3 μCi ^{203}Hg
- 2 100% uptake
- 3 10% in the cerebellum 54% in the liver
36% in the other parts of the body
- 4 Biologic half life 83 days for the cerebellum

and 71 days for the other organs

- 5 Weight of cerebellum 150 g, of the liver 1 700 g
and of the whole body 70 kg

The resulting doses were therefore, 600 mrad to the cerebellum, 300 mrad to the liver and 11 mrad to the rest of the body. If the activity had been distributed in other parts of the brain, then the dose to the cerebellum would have been less and similarly for the liver. Thus the doses estimated for the cerebellum and the liver are expected to be the maximum possible doses.

The experiments were carried out on three research workers who have detailed knowledge of isotope work and were thus able to judge both the radiation and the toxicity risks involved in the experiments. Therefore no ethical radiation problems arose in this case. In other cases, however, special attention must be paid to such problems. According to normal practice in Sweden, a subject must actively volunteer, and further, have the understanding and the background to be able to assess the possible risks to which he may be exposing himself. At several of the larger hospitals isotope committees have been set up in order to facilitate the appraisal of these risks. Each investigation in which human subjects are used, even such studies which can be classed as non routine diagnosis or therapeutic treatment, or which are undertaken not only for the good of the subject (patient) have to be appraised and approved of by such a committee. As a general rule, total doses which are less than the permitted weekly dose for personnel engaged in radiologic work according to the ICRP, are considered to be insignificant from the point of view of health risks.

Effects noted during calibration For calibration, phantoms were used which consisted of a number of 500 ml plastic bottles containing a solution of methyl ^{203}Hg . In order that the bottles should be interchangeable, the quantities contained in them were accurately equal. This was assured partly by accurate pipetting and also by measuring the radioactivity in each bottle. However, it was noted that after a certain period of time, the amount of ^{203}Hg in the bottles varied, and after further check measurements it could be shown that, when the radioactive decay had been corrected for, the amount of ^{203}Hg had decreased by a half after approximately 4 months. As leakage of the solution could be discounted, the only feasible explanation is that methyl mercury diffuses through the polyethylene walls of the bottles.

The effect observed here can have serious consequences if, for example, polyethylene bottles are used to contain standard samples of methyl ^{203}Hg . Further, there is considerable risk of contamination of adjacent areas and personnel. It may be mentioned that inorganic mercury in solution does not diffuse through the walls of polyethylene containers.

Acknowledgements

This work has been carried out at the National Institute of Radiation Protection Stockholm and the authors acknowledge humbly their indebtedness to the director Professor Bo Lindell for his interest and support. The authors are greatly indebted to Mr Gunnar Eklund who has performed the measurements and given valuable assistance in the work.

SUMMARY

Distribution measurements were made using a whole body counter on three subjects who had taken $26 \mu\text{Ci CH}_3^{203}\text{Hg NO}_3$ orally. Profile curves showed that the uptake was specifically concentrated to two regions in the body which correspond to the brain and the liver. The biologic half life was calculated. The various sources of error are discussed and conclusions drawn concerning their effect on the final results. The radiation doses were estimated and were found to be acceptable. The results reported concern specifically the three subjects involved in the present series of measurements but it may be assumed that these results are also applicable to other clinically healthy men 35 to 45 years of age.

ZUSAMMENFASSUNG

Bei drei Personen die $26 \mu\text{Ci CH}_3^{203}\text{Hg NO}_3$ oral eingenommen hatten wurde die Körperverteilung unter Anwendung eines Ganzkörper Zählors bestimmt. Aus den Profilkurven geht hervor dass die Aktivität spezifisch in zwei Körperregionen und zwar dem Gehirn und der Leber konzentriert wird. Die biologische Halbwertszeit wurde berechnet. Die verschiedenen Fehlerquellen werden diskutiert und die Folgerungen daraus für die Endresultate gezogen. Es wurden die Bestrahlungsdosen bestimmt die sich als acceptabel erwiesen. Die mitgeteilten Resultate gelten für die drei Personen der vorliegenden Messserie aber kann angenommen werden dass diese Ergebnisse auch für andere klinisch gesunde 35 bis 45 jährige Männer gültig sind.

RÉSUMÉ

Des mesures de distribution de dose ont été faites au moyen d'un compteur corporel total sur trois sujets qui avaient pris par voie buccale $26 \mu\text{Ci CH}_3^{203}\text{Hg NO}_3$. Les courbes de profil ont montré que la fixation était concentrée sélectivement dans deux régions du corps qui correspondent au cerveau et au foie. Les auteurs ont calculé la demi-vie biologique. Ils examinent les différentes causes d'erreur et tirent des conclusions concernant leur influence sur le résultat final. Ils ont estimé les doses de radiation et les ont trouvées acceptables. Les résultats présentés concernent spécifiquement les trois sujets soumis à cette série de mesures mais on peut supposer que ces résultats sont aussi applicables à d'autres hommes en bonne santé âgés de 35 à 45 ans.

REFERENCES

- ÅBERG B, EKMAN L, FALK R et coll. Excretion and distribution of ^{203}Hg administered as monomethylradiomercury to human volunteers. To be published in Arch environm Hlth.
- FKMAN L, GREITZ U, PERSSON G och ÅBERG B. Omsättning av metylkvicksilver hos människor (In Swedish) Nord Med 79 (1968) 150.
- — MAGI A et coll. Fördelning av kvicksilver 203 efter tillförsel av metylradiokvicksilvernitrat (In Swedish) Nord Med 79 (1968) 456.
- LINDILL B and MAGI A. A new laboratory for whole body counting. In Proceedings of the First Nordic Radiation Protection Conference Acta radiol (1966) Suppl No 254 p 135.
- MAGI A. Strålskyddsinstitutets Högaktivitetslaboratorium för helkroppsmätning. Beskrivning och kalibrering samt redogörelse för vissa jämförande mätningar (In Swedish) Statens strålskyddsinstitut (1967) Report No 18.

HIGH ENERGY ELECTRON BEAM TREATMENT PLANNING FOR BETATRON 10—24 MeV

by

RICHARD F NELSON

Historically the first successful operation of the betatron was accomplished by KERST at the University of Illinois in 1940. Earlier attempts were made by WIDERÖF of Germany, WALTON in England and BREIT & TUVE in the United States. Although the concept of the equilibrium orbit was known before KERST's work, the careful theoretical study of the conditions for particle injection made by KERST & SERBER resulted in making the betatron a practical instrument.

In 1945 KERST proposed a method for extracting electrons from the betatron using a magnetic shunt. Using this method HARVEY HASS & LAUGHLIN (1951) extracted electrons from the betatron at the University of Illinois Medical School and proceeded with their clinical application. The principles of high energy electron beam treatment planning were then initiated and developed. Emphasis was placed on the full utilization of the machine's versatility, with special attention paid to such parameters as electron energy, field size, differential attenuation with wax and plaster alignment casts and molds. This was the first application of high energy electrons to patients.

In 1953 an Allis-Chalmers electron beam donut with magnetic shunt and other associated apparatus was assembled at the Memorial Center in New York.

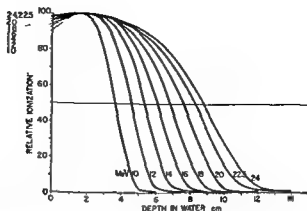


Fig 1 Central axis depth dose data
electron beam field size 6 cm \times 8 cm

in preparation for the inception of electron beam therapy LAUGHLIN et coll used this technique for extracting high energy electrons from the Memorial betatron, thus making this the second institution to use electrons clinically Although there are many betatrons in use today, the Memorial Center to date probably has the largest series of electron beam patients in the world

The energy was originally determined by adjusting the strength of the magnetic field and expanding the electrons always at the time of maximum field strength However, in the newer model machines, the strength of the magnetic field is not altered, it is always run to maximum The energy of the electrons is determined by the time at which the electrons are suddenly expanded out of the equilibrium orbit This modification was done as it was felt a more mono energetic beam of electrons could be obtained The energy spread in the original technique was approximately 0.1 MeV

The roentgen ray electrons are extracted in the first quarter cycle and after expansion exit on the right of the machine after hitting a platinum pin target The electron beam is expanded in the third quarter and extracted through the left side of the machine where there is no target, i.e. the electrons are travelling in the opposite direction The electrons subsequently escape through a thin window in the donut in a rectangular configuration Scattering foils are used to disperse the electrons sufficiently to produce a uniform field The foils are made of high atomic number material (such as lead) so as to achieve maximum electron deflection with low roentgen ray contamination

The electron beams are monitored by a parallel plate ionization chamber This chamber consists of three dural plates supported in a lucite framework with a 2 mm plate separation The collection voltage is applied to the two outside plates and the center plate is connected to an amplifier In the electron mode

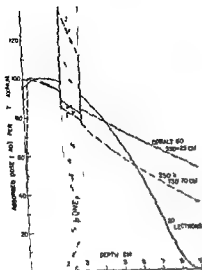


Fig 2 Central axis depth dose data for 250 kV cobalt and 20 MeV electrons corrected for inhomogeneity (bone) field size 10 cm \times 10 cm

one obtains about 300 R per minute at a meter on the Allis-Chalmers betatron

Both the energy and the output of the electron beams are calibrated. The energy of the electron beam is calibrated using the electro-disintegration threshold of ^{63}Cu which is 10.9 MeV and ^{12}C which is 18.7 MeV. The copper and carbon foils are exposed to the electron beam. The resultant positron emitting isotopes are counted with a scaler and these two points plotted on the linear calibration curve of the expander circuit. By interpolation and extrapolation various energies can be determined.

The output is calibrated using a 25 R Victoreen chamber placed in a polystyrene block at a depth of 1.5 cm. Using the cobalt 60 correction factor the machine is calibrated for the number of R per count at 20 MeV for a 10 cm \times 10 cm field. This is then related to the absorbed dose in rad in patients by the use of radiation chemical dosimeters or by calorimetric methods.

One and a half inch lucite is used to collimate the electron beam so as to minimize the production of scatter. The cones are constructed in such a manner as to allow the use of inserts for special field shaping. The ends of these cones are beveled in order to permit close contact with the skin surface. For areas where it is difficult to get good contact with the skin surface due to the thickness of the lucite cones one or more of the walls can be replaced by 1 cm thick aluminum.

Depth dose measurements are obtained with air cavity ionization chambers

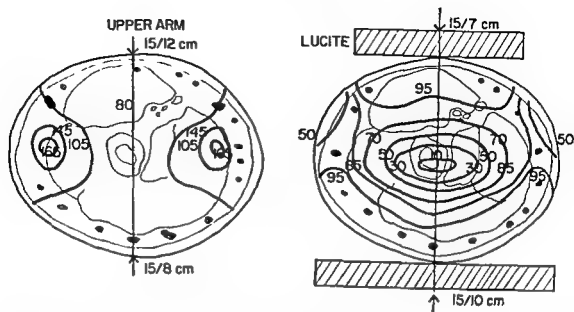


Fig 3 Dose distribution obtained from opposed fields without and with lucite plates 10 MeV betatron electron beam Hodgkin's disease

or P-N junction diodes immersed in a water phantom. Central axis dose measurements were made for energies from 10 MeV to 24 MeV with the Allis-Chalmers betatron, and for a variety of circular, rectangular, and square fields. The measurement of dose distributions is accomplished by attaching the same kind of air cavity chamber and water phantom to an automatic isodose recorder which automatically plots the dosage points from the various chamber positions. Dose distributions were made for both beam axes, parallel to the donut and perpendicular to the donut. The greatest variation in the resulting distribution is found in the plan parallel to the donut and for high energies. This variation is caused by the geometry of the magnetic shunt. The maximum variation in depth dose with field area from 50 cm² to 225 cm² using these techniques is only 5%.

High energy electrons are not used for their skin sparing effect as are high energy roentgen rays. Although the dose to the skin varies with field size and energy, the maximum skin sparing is approximately 10%. The central axis depth doses for a field 6 cm × 8 cm for energies from 10 to 24 MeV are given in Fig 1. As may be seen from the diagram, the main advantage of high energy electron beam therapy is the uniform dose it produces in the first few centimeters of tissue, and the subsequent very sharp fall off of that dose. The amount of bremsstrahlung contamination in these energy ranges is between 2 to 4%. If one were to increase the energy much above 35 MeV, the main advantage of

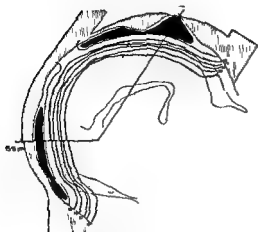


Fig 4 Dose distribution for subcutaneous metastasis to skull 10.4 MeV betatron electron beam

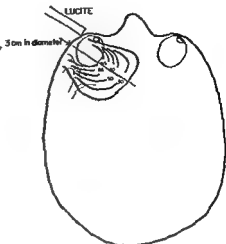


Fig 5 Dose distribution for choroidal metastasis 10 MeV betatron electron beam

high energy electron therapy would be lost because the increase in scattering at these higher energies causes the slope of the depth dose to become much less steep.

There is no preferential bone absorption per gram of tissue in these energy ranges. This makes it possible easily to correct the corresponding dose distribution for the increased density effect. A useful criterion has been found to allow 2 cm unit density absorption to 1 cm bone absorption. In Fig 2 are presented the absorbed doses (rad) produced by the absorption of a conventional 250 kV (30 mm Cu HVL) roentgen ray beam, a cobalt 60 gamma ray beam, and the 20 MeV betatron electron beam for a heterogeneous medium water and bone.

In view of such phenomena as sharp fall off and differential increase in depth dose with energy, the tumor depths treated with electrons in these energy ranges are limited. One would not treat a patient with a single field if the tumor depth were greater than 7 cm, nor would one treat a patient with opposed fields if the diameter were greater than 17 cm.

A dose distribution from a high energy electron beam is unique in that it does not fall off according to the inverse square law. The relative lack of divergence of the beam, which is caused by the scattering foils creating a uniform field, is of great advantage in treatment planning. In patients where the contour is rapidly changing, such as post surgical defects, there is no correction for the inverse

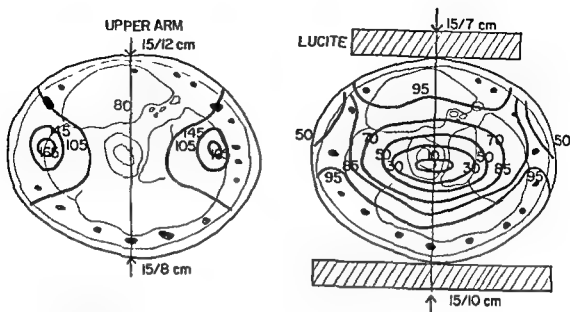


Fig 3 Dose distribution obtained from opposed fields without and with lucite plates 10 MeV betatron electron beam Hodgkin's disease

or P-N junction diodes immersed in a water phantom. Central axis dose measurements were made for energies from 10 MeV to 24 MeV with the Allis-Chalmers betatron, and for a variety of circular, rectangular, and square fields. The measurement of dose distributions is accomplished by attaching the same kind of air cavity chamber and water phantom to an automatic isodose recorder which automatically plots the dosage points from the various chamber positions. Dose distributions were made for both beam axes, parallel to the donut and perpendicular to the donut. The greatest variation in the resulting distribution is found in the plan parallel to the donut and for high energies. This variation is caused by the geometry of the magnetic shunt. The maximum variation in depth dose with field area from 50 cm² to 225 cm² using these techniques is only 5%.

High energy electrons are not used for their 'skin sparing' effect as are high energy roentgen rays. Although the dose to the skin varies with field size and energy, the maximum skin sparing is approximately 10%. The central axis depth doses for a field 6 cm \times 8 cm for energies from 10 to 24 MeV are given in Fig 1. As may be seen from the diagram, the main advantage of high energy electron beam therapy is the uniform dose it produces in the first few centimeters of tissue, and the subsequent very sharp fall off of that dose. The amount of bremsstrahlung contamination in these energy ranges is between 2 to 4%. If one were to increase the energy much above 35 MeV, the main advantage of

Table
Comparative values in treatment planning

	Supervoltage cobalt 60	Orthovoltage 250 kV	High energy electrons
1 Depth dose	Non uniform	Non uniform	Uniform
2 Preferential absorption in bone	None/g	Yes	None/g
3 Fall off ratio (tumor/normal)	Poor	Good	Excellent
4 Film dosimetry	Good-excellent	Poor	Excellent
5 Ease of field wedging	Difficult	Difficult	Very easy

with a pinhole for the patient to look through while the opposite eye is being treated insures proper rotation of the eyeball

It has long been the opinion of many people in the field of radiologic physics that the drawback of lack of skin sparing could be overcome by using grids. To date very little work has been done on it outside of the work of Jacques Ovadia at Michael Reese Hospital in Chicago. The phenomenon is that due to scatter below the surface one attains a uniform dose although the center of the field has been blocked out. Therefore by making a series of open and closed areas adjacent to one another one can, in effect, obtain a quasi skin sparing effect. On this premise a treatment cone jig has been designed by which one may use any combination of open and closed areas to fit the isodose to literally any patient in any circumstance undergoing radiation therapy.

In summary the betatron in the electron mode is a highly useful tool in the treatment of patients undergoing radiation therapy where one is able to use single fields where tumor is located at a depth not exceeding 7 cm or where the diameter of the patient in the treatment area is not greater than 17 cm. The comparative values that have to be considered in the treatment planning for patients may be judged from the data presented in the accompanying Table.

Acknowledgements

The author wishes to thank Dr. John S. Lau, M.D., of Memorial Hospital, New York, for his invaluable help and direction over the years, where most of this work was originally done. He also wishes to thank Barbara Edgar for her assistance with the figures.

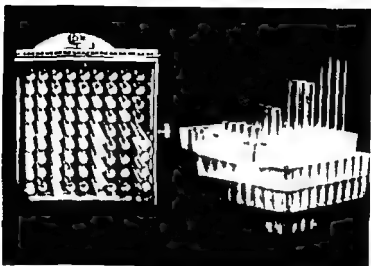


Fig. 6 Lucite inserts for treatment cone showing various plates and rods for shaping resultant isodoses

square law. These assumptions have been checked using film dosimetry and appear to be quite accurate.

The use of polystyrene, lucite or tissue equivalent wax, has been found extremely useful for shaping the electron beam. Most head and neck patients treated by the betatron have plaster alignment casts made and, if the initial treatment plan shows the existence of a hot spot, a wax wedge can easily be formed on the cast.

The betatron in these energy ranges is particularly useful in the treatment of such areas as breast, nasopharynx, substernal mass, and mammary lymphatic drainage points. Also for areas where one desires to avoid such structures as the spinal cord, and yet deliver a uniform dose to cervical nodes.

The use of lucite plates to increase the field separation in opposing fields is a convenient method of obtaining a low midplane dose and a high peripheral dose. Fig. 3 shows an upper arm distribution with and without lucite plates.

A difficult problem in treatment planning pertains to subcutaneous metastases to the skull. The combination of two oblique fields and wax absorbers with a designed shape to attenuate the beam differentially can produce a uniform subcutaneous dose with minimal brain dose, as seen in Fig. 4.

Another problem case in the treatment plan is choroidal metastases, and betatron electrons offer an ideal solution. One method is exemplified in Fig. 5. By placing a lucite wedge in the cone, it is possible to avoid treating the lens. A cast

PROGNOSIS IN HODGKIN'S DISEASE WITH SPECIAL REFERENCE TO HISTOLOGIC TYPE

Results of treatment predominantly by cytostatics

by

A. P. ANDERSEN, H. BRINCKER and F. LASS

Hodgkin's disease has been considered by some authors to be of inflammatory or immunologic origin but today it is generally accepted by clinicians as well as by pathologists that the disease belongs to the group of malignant lymphomas. It has been widely considered a multifocal systemic disease because of the systemic symptoms but more recent investigations (ROSENBERG & KAPLAN 1966, KAPLAN 1966) have indicated that the disease can just as well be considered of unifocal origin spreading by continuity.

The results obtained by intensive radiotherapy in various American and British clinics and presented at the Symposium in Rye, New York, in 1966 (Cancer Res. vol. 26 pp. 1034—1311) seem to accord with the unifocal theory and thereby with curability. The material now presented is therapeutically charac-

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SUMMARY

A brief review of the historical development of the use of high energy electrons is presented. Various examples of treatment plans showing the versatility of this modality are given. A table showing the comparative advantages and disadvantages of this method of treatment versus cobalt 60 and 250 kV is also presented.

ZUSAMMENFASSUNG

Eine kurze Übersicht über die historische Entwicklung der Verwendung hochenergetischer Elektronen wird gegeben. Verschiedene Beispiele für Behandlungspläne, die die Vielseitigkeit dieser Modalität zeigen, werden angeführt. Eine Tabelle, die die Vorteile und Nachteile dieser Methode vergleichend mit der Cobalt 60 und 250 kV Therapie zeigt, wird ebenfalls gegeben.

RÉSUMÉ

L'auteur fait un bref rappel de l'histoire de l'emploi des électrons de haute énergie. Il donne divers exemples de plans de traitement de façon à montrer la souplesse de cette méthode de traitement. Il présente aussi un tableau les avantages et les inconvénients de cette méthode de traitement par rapport au cobalt 60 et à la roentgen thérapie de 250 kV.

REFERENCES

- CHU T, NISCE L: Electron beam therapy of cancer of the breast. *Radiology* 89 (1967), 2116
 HARVEY R. A., HAAS L. L. and LAUGHLIN J. S.: Preliminary clinical experience with betatron. *Radiology* 56 (1951) 394
 KERST D. W.: A 20 million electron volt betatron or induction accelerator. *Rev. sci. Instr.* 13 (1942) 387
 LAUGHLIN J. S.: Physical aspects of high energy electron therapy. *Radiology* 99 (1967) 915
 — High energy treatment planning and dosimetry: Past experience and future development. *Front. radiat. Ther. Onc.* 2 (1968) 87
 — Realistic treatment planning. *Cancer* 22 (1968) 716

Clinical staging was made according to the following three groups

- Stage I* Disease restricted to one lymphatic region or to two adjacent lymphatic regions on the same side of the diaphragm
- Stage II* Disease involving more than two lymphatic regions or two lymphatic non adjacent regions on the same side of the diaphragm
- Stage III* Disease involving the lymphatic system on both sides of the diaphragm or other organs

All stages were also subdivided into clinical types A or B indicating the absence or presence of systemic symptoms i.e. fever pruritus or an abnormal tendency to sweating

The staging thus differs somewhat from that of PETERS (1950), and PETERS & MIDDLEMISS (1958) as well as that of WESTLING (1965). The classification in four stages (ROSENBERG 1966) which by now is internationally recognized could not be used *inter alia* because only a few of the patients had undergone lymphangiography. However stages I and II are identical with the corresponding stages of the named classification.

Histologic classification The histologic classification suggested by the Nomenclature Committee (LAKES et coll. 1966) was used in the review of the preparations: lymphocytic predominance, nodular sclerosis, mixed cellularity and lymphocytic depletion.

A group of ten cases of possible Hodgkin's disease had also to be included. Diagnostic Reed-Sternberg cells could not be demonstrated on review of these cases but in all other respects the histologic appearances were extremely suggestive of Hodgkin's disease and the cases could not be classified as non-specific reactions or as malignant lymphomas of a different nature.

Remissions Remission after a given treatment is taken to mean objective improvement with subsidence of pathologic lesions or subsidence of systemic symptoms with or without disappearance of the lesions. No distinction could be made between partial and total remissions.

Results

Age and sex distribution The distribution of the material by age and sex is given in Fig. 1. In both sexes there is, as in other materials, a preponderance of patients in the age group 20 to 29 years. No regard was however paid to the age

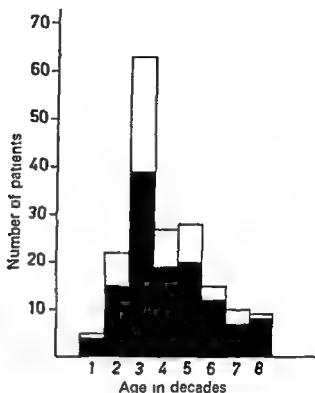


Fig. 1 Distribution of the material according to age and sex: females (55) indicated by white columns and males (124) indicated by black spaces

terized by previous views holding that Hodgkin's disease is a fatal systemic disease and that the main emphasis must be on chemotherapy. The object of the study has been to report our results and in particular to correlate them to the histologic classifications of the Rye conference.

Material and Method A total of 195 cases diagnosed as Hodgkin's disease were treated during the period 1st of January 1955 to 31st of December 1964. The histologic preparations in seventeen of these cases could not be procured but in all the others they were reviewed and reclassified by one of the present authors (T. L.) without knowledge of the clinical course.

Ten cases in which the diagnosis could not be maintained were excluded. Of the unreviewed cases, those in which the histologic report did not mention typical Reed-Sternberg cells were also excluded, i.e. a further six cases. This left 179 cases and in eleven of these the histologic preparations were not reviewed. The material was analyzed as of 31st December 1967 so that the follow-up period was from 3 to 13 years, the follow-up rate being 100%. A total of 115 cases were treated primarily in the clinic, while 64 were treated secondarily, the primary treatment having been given elsewhere. All survival times have been calculated from the time at which a definite diagnosis was made.

Table 3

Distribution by histologic types compared with the histologic groups of Lukes et coll

Histologic type	Present material*		LUKES BUTLER & HICKS (1966)		Histologic groups
	Number of cases	Per cent	Number of cases	Per cent	
Lymphocytic predominance	24	17	■	17	L. & H. nodular L. & H. diffuse
Nodular sclerosis	46	32	149	40	Nodular sclerosis
Mixed cellular type	48	34	97	25	Mixed
Lymphocytic depletion	24	17	68	18	Diffuse fibrosis Reticular
Total	142	100	377	100	

* The groups possible Hodgkin's disease (15 cases) non specific changes (at first biopsy) (4 cases) and no revision (18 cases) excluded

distribution of the general population since a bimodal age specific distribution would then be expected with an accumulation of cases between 20 to 29 years and over 60 (MEIGHAN & RAMSAY 1963)

There were 124 males and 55 females in the material, which gives a male/female ratio of 2.26 : 1. This is somewhat higher than generally reported i.e. ROOS & VIDEBAEK (1959) found 1.06 : 1. MÜSSHOF & BOUTIS (1968) 1.3 : 1. WESTLING (1965) 1.58 : 1 while MEIGHAN & RAMSAY (1963) found 2.06 : 1, and O'BRIEN & O'BRIEN JR (1954) 3.0 : 1.

Distribution by clinical stage The distribution by clinical stage at the time of diagnosis is presented in Table 1. In 22 cases it was not possible on the basis of the available data to make any clinical staging and in a further 16 cases it was not possible to sub-classify the cases into A or B types. While for the material considered as a whole there is a fairly equal distribution within the three clinical stages there seems to be a female preponderance in stage II and a male preponderance in stages I and III. As might be expected there is a marked increase of the systemic symptoms in the more advanced clinical stages.

Distribution by histologic type This is presented in Table 2 based upon the revision of the first biopsy specimen ever taken. As may be seen nodular sclerosis was more common in females while mixed cellularity occurred more often in males. Moreover lymphocytic predominance was found to be extremely rare in women.

Table 1
Distribution by clinical stage systemic symptoms and sex

Clinical stage	Females			Males			Total females	Total males	Total females + males
	A	B	Σ*	A	B	Σ*			
I	9	1	2	35	4	1	12 (22 %)	40 (32 %)	52 (29 %)
II	17	5	5	11	12	4	27 (49 %)	27 (22 %)	54 (30 %)
III	0	0	0	6	32	4	0	42 (16 %)	51 (34 %)
Unknown			7			15	7 (13 %)	15 (12 %)	22 (12 %)
Total	26 (47 %)	15 (27 %)	14 (26 %)	52 (42 %)	48 (39 %)	24 (19 %)	55 (100 %)	124 (100 %)	179 (100 %)

* Unknown whether systemic symptoms were present

Table 2
Distribution by sex and histologic revision of first biopsy

Histologic type	Females		Males		Total	
	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent
Lymphocytic predominance	1	2	23	18	24	13
Nodular sclerosis	20	37	26	21	46	26
Mixed cellularity	17	31	31	25	48	27
Lymphocytic depletion	9	16	15	12	24	13
Possible Hodgkin's disease*	3	5	12	10	15	9
Non specific changes**	1		3		4	
No revision***	4	9	14	14	18	12
Total	55	100	124	100	179	100

* By histologic revision of later biopsy five cases classified according to LUKES et coll (See table 4)

** Histologic revision of later biopsy indicated Hodgkin's disease and classification was made according to LUKES et coll (See table 4)

*** No histologic revision of first biopsy was made but in seven cases a later biopsy was revised and classified according to LUKES et coll (See table 4)

Table 5

Relationship between histologic types and clinical stage

Histologic types	Number of cases	Per cent of cases in the different clinical stages		
		I	II	III
Lymphocytic predominance	21	52	29	19 *
Nodular sclerosis	44	36	43	21
Mixed cellularity	44	32	34	34
Lymphocytic depletion	21	19 *	29	5*
Possible Hodgkin's disease	11	36**	36*	28*

† This group contains only 3 cases

* This group contains only 4 cases

Relation between clinical stage and histologic type The clinical stage and the histologic type on the first biopsy are compared in Table 5. Only cases in which the clinical stage could be assessed have been included in this comparison. It is seen that there is a preponderance of the type lymphocytic predominance in stage I decreasing towards clinical stage III. Lymphocytic depletion correspondingly showed a preponderance of cases in clinical stage III decreasing towards clinical stage I. Nodular sclerosis and mixed cellularity, the intermediate histologic types, and the group of possible Hodgkin's disease exhibit a more even distribution between the three clinical stages.

The material of LUKES, BUTLER & HICKS had exactly the same tendency as regards the distribution within the clinical stages. However these authors reported a somewhat greater preponderance of the histologic groups lymphocytic or histiocytic proliferation, nodular + diffuse within clinical stage I (70%), but the clinical stages used were not entirely identical. The groups diffuse fibrosis and reticular were together most common in stage III (56%), while nodular sclerosis and mixed cellularity were approximately equally represented in the three clinical stages.

Survival in the entire material Fig. 2 gives the percentage survival in the entire material: a 5 year survival of 33.1% and a 10 year survival of 13%. These findings agree with those of others in similar series in which radiotherapy and cytostatic treatment has been employed (SUTSKIN et coll 1955, TRUBSTEIN 1956, ROOS & VIDERBAEK 1959 and COOK et coll 1959). However these results do not appear to be up to those obtained by PETERS et coll (1966) by intensive radiotherapy, at least not as regards the 10-year survival.

Table 4

Evolution of the histologic process in the present series

Primary histologic diagnosis	Secondary histologic diagnosis						
	Non specific changes	Possible Hodgkin's disease	Lymphocytic predominance	Nodular sclerosis	Mixed cellularity	Lymphocytic depletion	No revision
Non specific changes (4 cases)			1			3	
Possible Hodgkin's disease (15 cases)		2	2			3	8
Lymphocytic predominance (24 cases)	2	1	4	1	1	4	11
Nodular sclerosis (46 cases)				10	2	10	24
Mixed cellularity (48 cases)				1	3	21	23
Lymphocytic depletion (24 cases)						13	11
No revision (18 cases)					1	6	11

It is of interest to compare the histologic distribution in the total series with that given by LUKES, BUTLER & HICKS (1966) in their material of 377 patients. They used a histologic classification which comprised six groups of which (1) lymphocytic and/or histiocytic proliferation, nodular, and (2) lymphocytic and/or histiocytic proliferation diffuse, are identical with the type lymphocytic predominance, while (5) diffuse fibrosis and (6) reticular are identical with lymphocytic depletion. The comparison is apparent from Table 3, in which cases that could not be classified on revision of the first biopsy are excluded, the percentage distribution has been corrected accordingly. The table indicates good agreement as to the distribution in the two materials.

Histologic reclassification on biopsies taken later in the course (33 cases) or on autopsy specimens (58 cases) in a total of 91 cases are listed in Table 4. In only four cases were the changes in the secondary histologic preparations less advanced than in the primary biopsies, while in forty four the histologic type was more advanced in the secondary specimens. Lymphocytic depletion was present in 54 out of the 58 autopsy specimens reviewed. The findings thus indicate that a change in the histologic appearances may occur in the course of Hodgkin's disease.

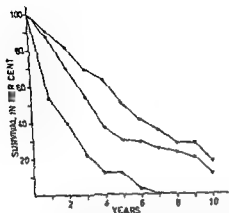


Fig 4 Survival (crude) rate in relation to the clinical stages I (57 patients ○—○) II (54 patients ●—●) III (51 patients □—□) (The stage was unknown for 22 patients)

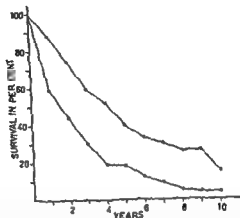


Fig 5 Survival (crude) rate in relation to systemic symptoms no symptoms (78 patients ○—○) systemic symptoms (63 patients ●—●) (It was unknown for 38 patients whether systemic symptoms were present)

After using intensive irradiation in the treatment of cases that he called localized lymphadenopathy EASSON (1966) reported a 5 year survival of 55 % and a 10-year survival of 43 % (age corrected survival rates) LAPLAN (1966) in a series treated by megavoltage irradiation gave a 5 year survival of 82.4 % for stages I and II a rate that remained unchanged until the 9th year (actuarial value)

A prognostic comparison of clinical types A and II is presented in Fig 5 which per se of course bear relation to the clinical stage (cf Table 1) Systemic symptoms constitute a serious prognostic sign (cf the above mentioned results of PETERS et coll for stage II A compared with stage II B) LUKES et coll (1966) even recorded poorer 5 year results for stage II B than for stage III A and HELLER et coll (1968) consider systemic symptoms in localized disease to be equally poor prognostically as lymphadenopathy and the dissemination in stage III

Survival in relation to histologic type Table 6 gives the percentage survival within the histologic types and for the group possible Hodgkin's disease at 1, 3, and 5 years. At 3 years there is a satisfactory correlation with a decreasing number of survivors in the more advanced histologic type. At 5 years on the other hand there is a striking fall in the number of survivors within the histologic type lymphocytic predominance as compared with the results of LUKES et coll (1966). This phenomenon will be discussed later.

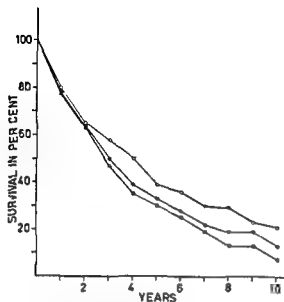


Fig 2 Survival (crude) rate in relation to sex: females (55 ○—○), males (124 □—□), entire material (179 patients ●—●)

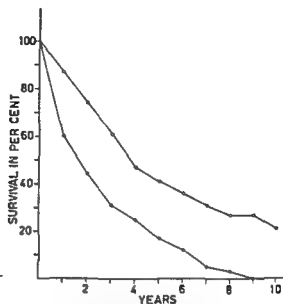


Fig 3 Survival (crude) rate in relation to age: under 40 years of age (117 patients ○—○) and over 40 years (62 patients ●—●)

Survival in relation to sex and age Fig 2 also gives the percentage survival for women and men separately. Like numerous other workers, the present authors found a somewhat better prognosis in women than in men, 39 % of the female patients living for 5 years as compared with only 30 % of the males; further more, 21 % of the female patients lived for 10 years, as compared with 7 % of the males.

The prognosis in relation to age is given in Fig 3. The material was divided into two age groups, patients over 40 and under 40. In the older age group the prognosis is distinctly poorer (5 year survival, 17 %) than in the younger (5 year survival, 41 %). This graph does not, however, take into account the higher normal mortality in the older age groups.

Survival in relation to clinical stage The percentage survivals in three clinical stages (22 cases excluded because of deficient data) are given in Fig 4. It is evident that the staging places the cases into three entirely different prognostic groups. The results are in keeping with those of WESTLING (1965) but for stages I and II there is a striking difference in relation to materials treated by more intensive irradiation, especially in respect to survivals beyond 5 years. Thus, PETERS et coll (1966) reported 5 and 10 year survivals for stage I of 73 % and 52 %, respectively, for stage II A 90 % and 67 % and for stage II B only 23 % and 13 %.

Table 6 (cont.)

		L. JONES BUTLER & HICKS (1966)		
5 years		5 years		Histologic groups
Number at risk	Survival per cent	Number at risk	Survival per cent	
22	27	63	73	L. & H. nodular
				L. & H. diffuse
36	44	149	44	Nodular sclerosis
40	15	97	37	Mixed
17	III	68	13	Diffuse fibrosis
				Reticular
13	69			

* The groups non specific changes (at first biopsy) and no remission excluded

(tumour dose) but the fields were generally rather small. Secondary radiotherapy was given with a purely palliative object, directed at local lesions causing symptoms.

Table 7 presents the primary treatment in relation to clinical stage. As might be expected, irradiation was most often used in clinically localized stages. If chemotherapy was used in localized stages, this was mainly in cases where the condition was characterized by systemic symptoms. However, chemotherapy without concomitant radiotherapy, in these stages is not in keeping with present views concerning the treatment of Hodgkin's disease (KARNOFSKY et coll 1963, AISENBERG 1964, KARNOFSKY 1966, PERRY et coll 1967).

Chemotherapy. From the total of 179 cases, 152 received at some stage between one to twelve courses of one or more out of a total of 15 cytostatic substances. Chemotherapy alone as the primary treatment (Table 8) was given to 56, while the others had the courses at later stages when the disease was more or less clinically advanced or generalized. A total of 468 courses were administered, but in some cases the cytostatic medication was combined with irradiation or with steroid agents so it was not possible to assess the cytostatic effect per se. Several cases moreover received two cytostatics in combination, but those used for this treatment varied so that for numerical reasons the combined treatment with cytostatics cannot be assessed. Lastly, several single courses were excluded because data for estimating the length of the remission were absent. This leaves 307 single courses that could be evaluated.

Table 6

Survival according to histologic types compared with the histologic groups of Lukes et coll

Histologic type	Present material*			
	1 year		3 years	
	Number at risk	Survival per cent	Number at risk	Survival per cent
Lymphocytic predominance	24	83	24	63
Nodular sclerosis	46	93	46	61
Mixed cellularity	48	71	48	38
Lymphocytic depletion	24	54	24	21
Possible Hodgkin's disease	15	93	15	100

Survival in relation to duration of symptoms before diagnosis In Fig 6, the material is divided into three groups by duration of symptoms. Sufficient data were however not available in 31 of the cases. There does not seem to be any definite difference between the three groups, so that in the present material it is not possible to assess the prognostic significance of the duration of symptoms.

Treatment

It is only natural that the treatment varied a good deal seeing that the material comprises a period of ten years. A number of cytostatic substances have been launched on the market during this period and used for such a short time on so few cases that it is impossible to draw conclusions. Most cases were treated several times by irradiation, cytostatics or steroids, and frequently these types of treatment have been used in varying combinations. Numerous factors are accordingly difficult to assess but an attempt will be made to point out a few features.

Irradiation About 160 to 250 kV radiation was used during the greater part of the period. Telecobalt was employed for only a few cases in the last years of the study period. The dosage varied a good deal but with a tendency to higher dosage in recent years. Cases of localized disease treated primarily by irradiation received a dosage generally in the range 1 500 to 2 500 R over two to three weeks.

Table 6 (cont.)

		LIKES BUTLER & HICKS (1966)		
5 years		5 years		Histologic groups
Number at risk	Survival per cent	Number at risk	Survival per cent	
27	27	63	73	L & H nodular
				I & H diffuse
6	44	149	44	Nodular sclerosis
40	15	97	37	Mixed
17	24	18	13	Diffuse fibrosis
				Reticular
13	69			

* The groups non specific changes (at first biopsy) and no remission excluded

(tumour dose) but the fields were generally rather small. Secondary radiotherapy was given with a purely palliative object directed at local lesions causing symptoms.

Table 7 presents the primary treatment in relation to clinical stage. As might be expected irradiation was most often used in clinically localized stages. If chemotherapy was used in localized stages this was mainly in cases where the condition was characterized by systemic symptoms. However chemotherapy, without concomitant radiotherapy, in these stages is not in keeping with present views concerning the treatment of Hodgkin's disease (KARVORSKI *et coll.* 1963, AISENBERG 1964, KARVORSKI 1966, PERRY *et coll.* 1967).

Chemotherapy. From the total of 179 cases 152 received at some stage between one to twelve courses of one or more out of a total of 15 cytostatic substances. Chemotherapy alone as the primary treatment (Table 8) was given to 56 while the others had the courses at later stages when the disease was more or less clinically advanced or generalized. A total of 468 courses were administered but in some cases the cytostatic medication was combined with irradiation or with steroid agents so it was not possible to assess the cytostatic effect *per se*. Several cases moreover received two cytostatics in combination, but those used for this treatment varied, so that for numerical reasons the combined treatment with cytostatics cannot be assessed. Lastly several single courses were excluded because data for estimating the length of the remission were absent. This leaves 307 single courses that could be evaluated.

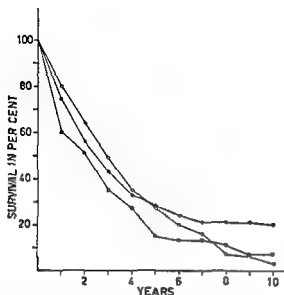


Fig 6 Survival (crude) rate in relation to duration of symptoms 0 to 5 months (88 patients ○—○) 6 to 11 months (37 patients □—□) over 12 months (23 patients ●—●) (Data missing for 31 patients)

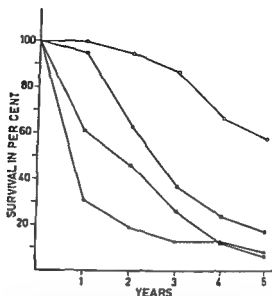


Fig 7 Survival (crude) rate in relation to duration of initial remission no remission (△—△) remission 1 to 6 months (□—□) remission 7 to 12 months (●—●) remission over 12 months (○—○)

The dependence of the prognosis upon the duration of the remission obtained by the primary treatment was analyzed regardless of whether it consisted of irradiation, chemotherapy, or a combination of both.

The result is presented in Fig 7. In the cases that obtained no remission at all after the initial treatment, the prognosis appeared to be serious, even at short sight, only 31 % surviving for a year. In the group of cases that obtained initial remission of from 1 to 6 months, only 6 % survived for 5 years, while the 5 year survival among the group having an initial remission exceeding 12 months was 58 %. The investigation thus indicates that the longer the initial remission the better the prognosis. A long initial remission may be a sign of slower progression of the disease, a factor that has been pointed out by Cook et coll (1959) and Karnofsky et coll (1963). Musshoff et coll (1966), on the other hand considered the remission rate and the duration of the first remission to be indications of therapeutic effectiveness.

In Table II are listed the six most common cytostatics representing 234 single courses without maintenance therapy. The remaining 73 courses are distributed over a total of nine additional cytostatics so that these numerical values are too small for comparison. The table also gives the average duration of the remissions with the drugs used. It will be seen that Natulan induced the longest

Table 7
Primary treatment according to clinical stage

Primary treatment	Clinical stage			Unknown	Total
	I	II	III		
Irradiation	8	28	12	14	99
Chemotherapy	6	18	29	3	56
Irradiation + chemotherapy	1	5	4	2	12
Miscellaneous types of treatment	7	3	0	3	19

remissions. The table also indicates the number of cases in which no remission was obtained. More than one course of the same drug was given in a number of cases. The duration of the remissions after the second and third courses was always shorter than after the first in all the cases in which more than two courses were available as a basis for the calculations. This indicates that secondary cytostatic therapy is less effective than primary therapy, a certain form of resistance developing on continued use of the same chemotherapeutic agent.

A comparison of the drugs triethylene melamine, cyclophosphamide, Natulan and vinblastine is presented in Fig. 8. The most striking feature is that Natulan and vinblastine give a larger number of remissions than the alkylating substances triethylene melamine and cyclophosphamide. In addition, it may be seen that with Natulan a larger number of long lasting remissions was obtained than with the other drugs. On the other hand, no remission lasted beyond 6 months on vinblastine. The alkylating agents triethylene melamine and cyclophosphamide possessed approximately the same effectiveness. With triethylene melamine especially there were only a few remissions longer than 6 months.

Discussion

Definite information concerning the spontaneous course of Hodgkin's disease is lacking as only very few series of untreated cases have been published. EWING in an early paper (1940) reported an average duration of 18 months. SHIMURA et al. (1955) collecting a total of 433 cases from the literature prior to 1936 reported a 5 year survival of less than 15% from the time of diagnosis. They compared this result with 1109 cases collected from the period after 1940 among which the 5 year survival was 26.6%. This difference is statistically significant. They mentioned that factors other than the treatment may have contributed to this difference but avoided drawing further conclusions.

Table 8

The six most frequently used drugs number of courses average duration of remission and number with no remission

Drug	One course	Two courses	Three courses	Four courses
Diepoxybutan	14	2	0	0
Triethylene melamine	85	26	7	1
Cyclophosphamide	26	5	1	0
Vinblastine	21	2	0	0
Vincristine	6	3	0	0
Natulan	29	6	0	0

At all events, the prognosis of Hodgkin's disease has improved in recent years, particularly in series where intensive radiotherapy has been used. The present results are in keeping with this trend.

Various factors influence the prognosis, inter alia the age. The prognosis is poorer in the older group of cases (Fig. 3), a factor explained by JACKSON & PARKER (1947) as a higher incidence of a sarcomatous histologic appearance, while UDDSTROMER (1934) believed that elderly patients had a greater tendency to generalization of the disease.

Sex is undoubtedly another prognostic factor (Fig. 2). However, doubts have been raised as to whether the apparently better prognosis in women is real. EPSTEIN (1939), PETERS et coll (1966) and WESTLING (1965) found the same sex difference but without a statistical significance and SHIMADA et coll (1955) reported a significant sex difference only for stage I. MEIGHAN & RAMSAY (1963) recorded a better prognosis only in women of fertile age, a phenomenon also pointed out by BICHEL (1955). VIDFRAEK (1950) could not demonstrate any sex difference, and MUSSHOF & BOUTIS (1968) believed that the better prognosis in women was due to a lower incidence of the more advanced clinical stages. The better prognosis in women in the present material may have been due to the lower incidence of clinically more advanced stages.

The clinical stage at diagnosis is an important prognostic factor (Fig. 4) and this seems to be generally accepted. Since the clinical stage no doubt bears some relation to the histologic appearance (Table 5), the latter must also be considered of importance, although, unlike LUKES et coll (1966), we failed to notice so benign a course of the histologic type known as lymphocytic predominance. Incidentally, it has been stated by HILTON & SUTTON (1962) that Hodg-

Table 8 (cont.)

Total number of courses	Average duration of remission in months				No remission	
	Total courses	First course	Second course	Third course	Number of courses	Per cent
16	14	11	(3.5)		8	50.0
119	22	25	15	16	41	34.4
32	29	34	0.8	(2.0)	11	34.4
23	30	30	(4.0)		3	13.0
9	34	43	1.7		1	11.1
35	50	57	2.0		2	5.7

lin's disease is the only group of malignant lymphomas in which clinical staging is of any value from the prognostic point of view.

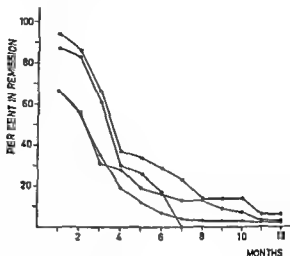
The duration of the disease before diagnosis (Fig. 6) has by some authors also been related to the prognosis. PETERS & MIDDLEMISS (1958) for instance regarded the 5 year survival to be the better the longer the disease had lasted before treatment. Later PETERS *et coll.* (1966) noted a considerable preponderance of 10-year survivors with symptoms for more than one year. WESTLING (1965) on the other hand reported the best 5 and 10 year survivals in cases with symptoms for only 0 to 6 months but the difference as compared to the other groups was not significant.

Finally the duration and the completeness of the remission on initial treatment is obviously of prognostic importance as is also apparent from Fig. 7.

During the past 50 years several attempts have been made to correlate the histologic appearances in Hodgkin's disease partly to the prognosis and partly to the anatomical spread of the disease. JACKSON & PARKER (1944) used a classification into three groups: paraganuloma, granuloma and Hodgkin's sarcoma. The first group represented a benign and slow course, the third group a malignant and short course. The main objection to this classification was that the group of paraganulomas made up only 7 to 8% of the cases and the group of Hodgkin's sarcoma only 2 to 3% while the intermediate group of granulomas represented about 90%. In this group therefore there was a wide histologic and thus also prognostic variation.

LEAKS *et coll.* (1966) originally used a classification comprising six groups which however was reduced to four types by the Nomenclature Committee. We felt that it might be of interest to test this classification by review and reclassification of the histologic preparations. This revealed conformity as regards the distribu-

Fig 8 Duration of remission following treatment with different drugs: Natulan (35 courses) ○—○; vinblastine (23 courses) ●—●; cyclophosphamide (32 courses) □—□; triethylene melamine (119 courses) △—△.



bution of the material within the histologic types (Table 3) and the distribution in relation to clinical stage (Table 5), with due consideration of the fact that our material was less than half as large as that submitted by LUKES et coll. The main disagreement was, as already mentioned, the 5 year survival in the group 'lymphocytic predominance' (Table 6). We cannot explain this disagreement. As emphasized by KELLER et coll. (1968) one of the difficulties with Lukes' classification is to distinguish between the types mixed cellularity and lymphocytic predominance. We may have placed some of the former group into the latter, and this would account for the discrepancy. This does not seem particularly likely, however, as the type 'lymphocytic predominance' in our material makes up exactly the same proportion (17%) of the total series as in that of LUKES et coll. On the other hand KELLER et coll. found this proportion to be only 5%. Another contributory cause of the poor prognosis with this histologic type in the present series may be that while only 52% of the cases in this group were in clinical stage I, LUKES et coll. had 70% in this stage. Furthermore, there was only one woman in the group, so that it is predominated entirely by the prognostically more unfavourable male patients. The favourable prognosis in lymphocytic predominance may be due to immunologic defence mechanisms, suppressed by the extensive use of chemotherapy so that the 5 year survival is poorer than that obtained by others who have used no radiation to a greater extent. This is of course mere hypothesis which cannot even be supported by the present findings. The histologic type nodular sclerosis has been emphasized by some workers (PERRY et coll. 1967) as the most difficult group to classify according to Lukes' system. Nevertheless we found conformity with the material of LUKES et coll. for this group as well as in respect to the 5 year survival.

We found that in a large number of the cases in which two biopsies had been performed in the course of the disease the histologic appearances had altered (see Table 4). This alteration was in a more malignant direction in the majority of the cases. However it is well known that different histologic Hodgkin changes within different groups of lymph nodes may be present in the same subject. Such differences may even occur within the same group of nodes or in the same histologic section (CUSTER & BERNHARD 1948, RUTTMER 1953). This may be the explanation of the change towards a more benign appearance that was noted in a few cases, a phenomenon also reported by CUSTER & BERNHARD. However the predominant number of cases with changes in the histologic appearances undergo a transformation in a more malignant direction. It may thus be expected that in a certain proportion of cases at least the clinical and histologic progression is concurrent. Histologic progression has also been demonstrated by CUSTER & BERNHARD (1948), JELLIFFE & THOMSON (1955), TRUBSTEIN (1956), and WESTLING (1965). It may be that other factors, e.g. bacterial, viral or fungal infections that often affect these patients in the terminal stages (CASAZZA et coll. 1966) may also be contributory. The same applies to a possible effect of irradiation and chemotherapy. However CUSTER & BERNHARD failed to demonstrate that irradiation alone could induce transformations in the histologic process either into a more malignant or a more benign direction (presupposing that the lesion had not healed during the treatment). Let it be emphasized that among the twenty-two cases of nodular sclerosis in which new biopsies were performed later in their course twelve presented evidence of conversion to more malignant histologic types, viz. two to mixed cellularity and ten to lymphocytic depletion. LUKES et coll. (1966) were unable to demonstrate with certainty any histologic evolution in nodular sclerosis but felt that it presumably existed.

It would appear that according to the view prevailing to-day isolated chemotherapy is not the treatment of choice for Hodgkin's disease in localized clinical stages I and II. Intensive radiotherapy is indicated in such cases. We must admit that the treatment of our material does not accord with these principles. However chemotherapy still remains of value for certain groups of cases in Hodgkin's disease. Although it is accepted that clinically less advanced stages of Hodgkin's disease may be cured by irradiation, a number of these cases will later become generalized and a not inconsiderable proportion have primarily been disseminated. In such instances chemotherapy is still of great value.

Chemotherapy may be administered in various ways, with or without irradiation, as courses of one drug with or without maintenance therapy, or as combination of two or more drugs.

The results obtained by chemotherapeutic management in our series (Table 8 and Fig. 8) cannot be compared directly with the results of others. In part

our results are based upon a retrospective study, not upon a controlled clinical series, and in part the definition of remission may vary. Within our own series, however, the various drugs may be compared, but for numerical reasons a relative assessment is permitted only for the individual drugs, not for combined medication.

Three factors must be stressed in evaluating the effectiveness of a cytostatic. In the first place the ability to induce remission and in the second place the duration of the induced remission must be considered. The third factor is the influence upon the patient's survival.

There is little doubt, on the basis of such a comparative evaluation of the cytostatics in the present series, that Natulrin was the most effective, both in respect to the duration and the frequency of remissions (Table 8). Moreover, Natulrin induced a relatively larger number of long lasting remissions, i.e. remissions sustained for up to one year (Fig. 8). Other authors (MATHÉ et coll. 1963, DE VITA et coll. 1956, SAMUEL et coll. 1967) have also experienced encouraging results in clinical experiments with Natulrin in Hodgkin's disease. Vinblastine and vincristine produced only a slight increase in the average duration of remissions, compared with the alkylating substances, but there was a marked increase in the incidence of remissions. Long lasting remissions were not common with vinblastine (Fig. 8).

As may be seen from Table 8, when the same drug was used there was a decrease in the average duration of remission in courses administered after the primary one and, as has been demonstrated repeatedly, resistance develops in the event of continued treatment with the same substance or group of substances. However, this need not entail cross resistance to other groups, which is often a difficult problem to assess, the secondary treatment usually being administered at a time when the disease is more advanced clinically as well as histologically and when the patient's general condition is poorer partly because of the disease and partly because of previous treatment which may have compromised the normal biologic defence mechanisms.

It is difficult to say whether cytostatic treatment, administered alone, prolongs survival in Hodgkin's disease, and the present material does not allow of any conclusions. SHIMKIN et coll. (1955) failed to demonstrate that HN (nitrogen mustard) was able to prolong life. Others (ROOS & VIDEBAK 1959, KARNOFSKY et coll. 1963, KARNOFSKY 1966) felt that life may be extended secondarily by improving the general condition through cytostatic therapy. However, it must be borne in mind that during the very period that cytostatics have been used a number of other therapeutic measures, such as modern antibiotics and corticosteroids, have been introduced and these too may constitute contributory aids. PERRY et coll. (1967) have expressed the most optimistic view in relation

to this problem stating that proper manipulation of the available chemotherapeutic substances may produce such destruction of the malignant cells that this per se prolongs the life of the patient

Conclusions

A 10-year material (1955—1964) comprising 179 cases of Hodgkin's disease is reported. Chemotherapy was used to a great extent but irradiation was employed as well, mainly for palliative purposes. The total 5 year survival rate was 33.1% and the 10 year survival rate 13%. The poorest prognosis was in (1) cases in an advanced clinical stage (2) cases with systemic symptoms (3) elderly patients (4) male patients and (5) patients with short and incomplete remission after the primary treatment. The duration of symptoms before the diagnosis did not appear to play any prognostic role.

All histologic preparations but eleven were reviewed and reclassification was made into the histologic types suggested by the Nomenclature Committee (Lukes et coll. 1966). There was agreement with the material of LUKES, BUTLER & HICKS (1966) in respect to distribution within the histologic type, relation to clinical stage and prognosis within the histologic types save the lymphocytic predominance type in which the prognosis was considerably poorer than reported by LUKES et coll. This finding is discussed in more detail. Signs of evolution in a more malignant direction of the histologic types with clinically progressing disease also within the type nodular sclerosis were apparent.

Natulan was the most effective of the various cytostatic drugs both in respect to the length of remission and the ability to induce remission.

Acknowledgements

The assistance of O. Kalhns, civil engineer in preparing the material for computer analysis is gratefully acknowledged. The work was supported by a grant from the Danish Anti Cancer League.

SUMMARY

A total of 179 cases of Hodgkin's disease treated predominantly by chemotherapy during the period 1955—1964 were reviewed histologically and reclassified into the histologic types recommended by the Nomenclature Committee (Lukes et coll. 1966). The 5 year survival rate was 33.1 per cent. Natulan® proved to be the most effective of the cytostatic drugs.

ZUSAMMENFASSUNG

In 179 Fällen der Hodgkin'schen Erkrankung, die hauptsächlich mit Chemotherapie während der Periode 1955—1964 behandelt wurden, wurden die Befunde histologisch überprüft und in die von der Nomenclature Committee (Lukes et coll. 1966) empfohlenen histologischen Typen reklassifiziert. Die 5-Jahre Überlebenszeit war 33.1%. Natulan® erwies sich als das beste der cytostatischen Mittel.

RÉSUMÉ

Les auteurs ont revu histologiquement et reclassifié d'après les types histologiques recommandés par le Comité de Nomenclature (Lukes et coll 1966) un total de 179 cas de maladie de Hodgkin traités principalement par chimiothérapie au cours de la période 1955—1964. Le taux de survie à 5 ans était de 33,1 pour cent. La Natulan® était le plus efficace des médicaments cytostatiques.

REFERENCES

- AISENBERG A. C. Hodgkin's disease — prognosis, treatment and etiologic and immunologic considerations. *New Engl J Med* 270 (1964) 508.
- BICHSEL J. Long remissions in Hodgkin's disease. *Acta radiol* 44 (1955) 325.
- CASAZZA A. R., DUVALLE C. P. and CARBONE P. P. Summary of infectious complications occurring in patients with Hodgkin's disease. *Cancer Res* 26 (1966) 1290.
- COOK J. C., KRABENHOFT K. I. and LEUCUTIA T. Combined radiation and nitrogen mustard therapy in Hodgkin's disease as compared with radiation therapy alone. *Amer J Roentgenol* 82 (1959) 651.
- CUSTER R. P. and BERNHARD W. G. The interrelationship of Hodgkin's disease and other lymphatic tumors. *Amer J med Sci* 216 (1948) 625.
- DEVITA V. T., SERPICK A. and CARBONE P. P. Preliminary clinical studies with ibenzmethyl. *Clin Pharmacol Ther* 7 (1966) 542.
- EASON E. C. Possibilities for the cure of Hodgkin's disease. *Cancer (Philad)* 19 (1966) 345.
- EPSTEIN E. Sex as a factor in the prognosis of Hodgkin's disease. *Amer J Cancer* 35 (1939), 230.
- EWING J. Neoplastic diseases: a treatise on tumors. Fourth edition. W. B. Saunders Co. Philadelphia 1940.
- HILTON G. and SUTTON P. M. Malignant lymphomas: classification, prognosis and treatment. *Lancet* 1962 I p. 283.
- JACKSON JR. H. and PARKER JR. F. Hodgkin's disease I. General considerations. *New Engl J Med* 230 (1944) 1.
- — Hodgkin's disease and allied disorders. Oxford University Press, New York 1947.
- JELLIFFE A. M. and THOMSON A. D. The prognosis in Hodgkin's disease. *Brit J Cancer* 9 (1955) 21.
- KAPLAN H. S. Role of intensive radiotherapy in the management of Hodgkin's disease. *Cancer (Philad)* 19 (1966) 356.
- Long term results of palliative and radical radiotherapy of Hodgkin's disease. *Cancer Res* 26 (1966) 1250.
- KARNOFSKY D. A. Chemotherapy of Hodgkin's disease. *Cancer (Philad)* 19 (1966) 371.
- MILLER D. G. and PHILLIPS R. F. Role of chemotherapy in the management of early Hodgkin's disease. *Amer J Roentgenol* 90 (1963) 968.
- KELLER A. R., KAPLAN H. S., LUKES R. J. and RAPPAPORT H. Correlation of histopathology with other prognostic indicators in Hodgkin's disease. *Cancer (Philad)* 22 (1968) 487.
- LUKES R. J., BUTLER J. J. and HICKS E. B. Natural history of Hodgkin's disease as related to its pathologic picture. *Cancer (Philad)* 19 (1966) 317.
- CRAVER L. F., HALL T. C. et coll. Report of the nomenclature committee. *Cancer Res* 26 (1966), 1311.

- MATHÉ G SCHWEISGUTH O SCHNEIDER M et coll Methylhydrazine in treatment of Hodgkin's disease *Lancet* 1963 II p 1077
- MEIGHAN S S and RAMSAY J D Survival in Hodgkin's disease *Brit J Cancer* 17 (1963) 24
- MUSHOFF K and BOUTIS L Therapy results in Hodgkin's disease Freiburg: Br 1948—1966 *Cancer (Philad)* 21 (1968) 1100
- — LASZLO A M und LASZLO I Prognostische Krankheitssymptome und zeichen bei Morbus Hodgkin und ihre Bedeutung für die Therapie der Erkrankung *Strahlentherapie* 131 (1966) 487
- O BRIEN F W and O BRIEN JR F W Hodgkin's disease *Amer J Roentgenol* 71 (1964), 1007
- PERRY S THOMAS L II JOHNSON R E et coll Hodgkin's disease Combined clinical staff conference at the National Institutes of Health *Ann intern Med* 67 (1967) 424
- PETERS M V A study of survivals in Hodgkin's disease treated radiologically *Amer J Roentgenol* 63 (1950) 299
- and MIDDLETON K C H A study of Hodgkin's disease treated by irradiation *Amer J Roentgenol* 79 (1958) 114
- ALISON R E and RAYMOND S B Natural history of Hodgkin's disease as related to staging *Cancer (Philad)* 19 (1966) 308
- ROOS D and VIDEBAEK A Prognosis in Hodgkin's disease *Dan med Bull* 6 (1959) 177
- ROSENBERG S A Report of the committee on the staging of Hodgkin's disease *Cancer Res* 26 (1966) 1310
- and KAPLAN H S Evidence for an orderly progression in the spread of Hodgkin's disease *Cancer Res* 26 (1966) 1225
- RUTTNER J R Zur pathologischen Anatomie der Lymphogranulomatose mit besonderer Berücksichtigung ihrer nosologischen Stellung *Med Habil Schrift Zurich Basel* 1953
- SAMUELS M L LEARY W V ALEXANIAN R et coll Clinical trials with N isopropyl- α (2 methylhydrazino) p-toluanamide hydrochloride in malignant lymphoma and other disseminated neoplasia *Cancer (Philad)* 20 (1967) 1187
- SHIMKIN M II OFFERMANN K C BOSTICK W L and LOW BEER B V A Hodgkin's disease An analysis of frequency distribution and mortality at the University of California Hospital 1914—1951 *Ann intern Med* 42 (1955) 136
- TATBESTEIN H Beitrag zur Behandlung der Lymphogranulomatose *Strahlentherapie* 99 (1956) 576
- Der differenzierte histologische Befund der Lymphogranulomatose als Grundlage des Behandlungserfolges *Strahlentherapie* 100 (1956) 62
- UDSTROMER M On the occurrence of lymphogranulomatosis (Sternberg) in Sweden 1915—1931 and some considerations as to its relation to tuberculosis *Acta tuberc scand* (1934) Suppl No 1
- VIDEBAEK A The course and prognosis of Hodgkin's disease *Acta med scand* 136 (1950) 203
- WESTLING F Studies of the prognosis in Hodgkin's disease *Acta radiol* (1965) Suppl No 245

EFFICACY OF PRIMARY RADIATION IN CARCINOMA OF THE ENDOMETRIUM

Preliminary study of unselected material from a specific region in the north of Sweden

by

O. KJELLGREN and S. S. MACNUSSEN

Carcinoma of the corpus uteri is notifiable in Sweden. Analysis of the Cancer Registry records (see ref. 27) reveals that endometrial carcinoma constitutes 6% of all malignant neoplasms in women. The annual incidence rate is 17 per 100 000 women, and 600 to 650 new cases are reported every year. The probability that a Swedish woman will develop endometrial carcinoma sometime during her lifetime is 1 in 66. It is a disease of later life and is rare in women under the age of forty (1%). In women over forty years of age, the incidence rate is 37 per 100 000 women. Between fifty-nine and seventy-nine years of age, the incidence rate is about 47 per 100 000 women, reaching a peak of 57 per 100 000 women in the 65 to 69 year group (Fig. 1). The age distribution in the 2 524 cases reported in Sweden during the period 1958—1961 is given in Fig. 2.

The management of carcinoma of the endometrium has for years been a subject of controversy all over the world. Two main lines of treatment are advocated: primary surgery with or without postoperative radiation, and primary

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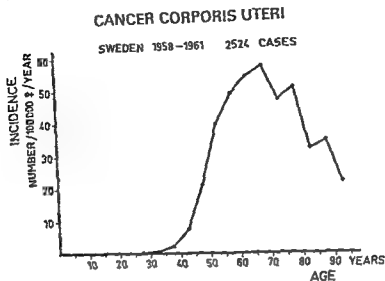


Fig 1 Incidence of endometrial carcinoma in different age groups in Sweden

radiation with or without supplementary operation (2-10 12-17 20-23 29-30) Most authorities now agree that primary surgery for early adenocarcinoma of the highly differentiated type gives excellent results it should be followed by vaginal vault irradiation in order to decrease the risk of vaginal recurrence This procedure reduces the incidence of recurrence from 12 to 3 or 4 % (3 16 24 28) In late cases with advanced changes management is more difficult These growths even if highly differentiated should probably be irradiated prior to operation In carcinoma of the undifferentiated type primary radiation followed by total hysterectomy and bilateral salpingo-oophorectomy is the treatment of choice The prognosis in anaplastic carcinoma treated by primary surgical resection is very poor even if the tumor is localized to the uterine cavity (17)

Evaluation of the clinical results of treatment of endometrial carcinoma is beset by many difficulties Although a vast literature has grown up around the subject the diversity of methods of management has made it difficult to collect a significant number of cases treated by any single technique Another difficulty is the matter of classification As KOTTMEIER (16) has pointed out there is no justification in comparing statistics based on surgically classified cases with those collected from clinically classified cases Finally and most importantly the problem of patient selection arises If the significance of this is disregarded or

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CANCER CORPORIS UTERI

SWEDEN 1958-1961 2524 CASES

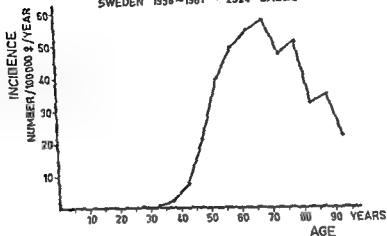


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Evaluation of the clinical results of treatment of endometrial carcinoma is beset by many difficulties. Although a vast literature has grown up around the subject the diversity of methods of management has made it difficult to collect a significant number of cases treated by any single technique. Another difficulty is the matter of classification. As KORTMEIER (16) has pointed out, there is no justification in comparing statistics based on surgically classified cases with those collected from clinically classified cases. Finally and most importantly the problem of patient selection arises. If the significance of this is disregarded or

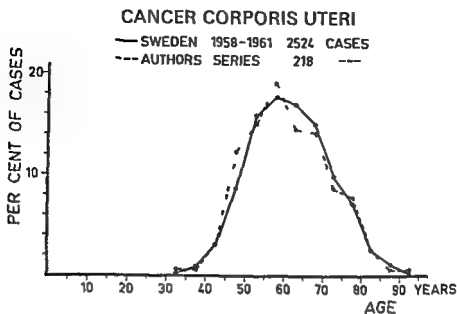


Fig. 2 Age distribution in the material compared to that of a larger Swedish series

minimized, unwarranted conclusions can too easily be drawn. For example, the results obtained with primary surgical resection followed by intravaginal radium are statistically excellent. It is, however, often overlooked that the case material on which these results are based is always highly selected, since it does not include cases of tumors that are either advanced or disseminated, nor those in which operation is contraindicated by concomitant disease. Furthermore, the criteria by which the cases are selected are often obscure, if given at all.

Many of the protagonists of primary surgery maintain that radiation is an ineffective form of treatment for endometrial carcinoma, pointing out that the 5 year recovery rates are much inferior to those obtained with primary surgery. A moment's reflection will reveal the fallacy in this reasoning. In most countries, the cases referred to radiotherapy centres are the unfavourable ones, those that have not been accepted for primary operation for the reasons stated above. In other words, the element of selection is again just as much in evidence, but this time the factors are weighted the other way. It should be stressed that the excellent results of primary radiation at Radiumhemmet have been achieved in a selected material since about 20 % of the cases referred to the institution have already been operated upon elsewhere. Incidentally, KOTTMEIER has never advocated radiation alone as the treatment of choice for endometrial carcinoma on the basis of these results, as has sometimes erroneously been maintained, but he advocates that operation should always be preceded by radiation.

With reservation for the obviously limited value of statistics, where the treatment of endometrial carcinoma is concerned the following figures give an idea of what can be achieved with different methods of management (19). Primary surgery followed by intravaginal radium, in selected cases of early malignancy confined to the endometrium or invading the inner half of the myometrium gives a 90 % 5 year recovery rate. In more advanced cases with invasion of the outer half of the myometrium it is about 60 % and in cases of pelvic recurrence treated with radiotherapy after primary surgery it is 40 %. The 5 year recovery rate for cases in stage I group 1 (clinically operable) treated with primary intracavitary radium with the Heyman packing technique alone is 85 %, the corresponding figure for stage I, group 2 (technically operable) cases is about 50 %. Cases which have been secondarily operated upon because of a recurrent growth have been included in these figures, the incidence of such operations is about 14 %. The recovery rate for cases operated upon because of failure of radiotherapy is about 60 % (ref 12—16).

GRAHAM writing in 1956 on the treatment of choice for carcinoma of the endometrium stated that he knew of no papers in which all the cases from a given geographic area were included. We are now able to present the preliminary results of such a study. Our department which has full radiologic and surgical facilities serves a specific region in the northern part of Sweden with about 700 000 inhabitants. There are no other radiotherapy centres in the area. All cases of possible or manifest endometrial carcinoma in this region are referred to the department for review and treatment. By arrangement with the gynecologic and surgical services within the area no therapeutic procedures are carried out on these cases prior to their admission to our department. The only exceptions are occasional cases in which endometrial carcinoma has been an incidental finding at hysterectomy for some other condition and which are thereafter referred to the department for postoperative radiotherapy. Thus therapeutic centralization has made it possible to carry out a uniform plan of management on an unselected material comprising all the known cases of endometrial carcinoma occurring in a specific region. The preliminary results of a therapeutic trial of this nature are presented in this paper. The efficacy of primary radiation for stage I and stage II endometrial carcinoma determined by the microscopic study of specimens obtained at major surgery or post radiation curettage is discussed. The term *primary cure rate* conveniently expresses the proportion of cases in which no residual malignancy was evident.

Material. Between 1963 and 1967, 242 cases of malignant tumors of the corpus uteri were treated. A total of 218 cases (90 %) had endometrial carcinomas and these make up the material. The remaining 24 cases (10 %) of

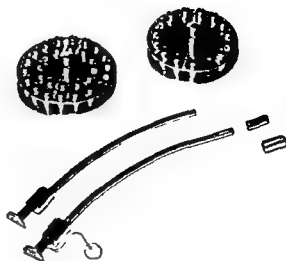


Fig 3 Instrumentarium used for intrauterine packing. Two jacket holders are seen uppermost, two applicators of which the lower one is loaded with an irradiator with thread and tag are seen below and to the right are two irridators of different size.

arcoma have been excluded from the study. The age distributions, in our material and in the 2 524 cases registered at the Swedish Cancer Registry during the period 1958—1961, are given in Fig 2. The material is unselected and is made up of all known cases of endometrial carcinoma occurring over a period of three years in the region served by the department. The staging of invasive carcinoma of the corpus uteri according to the international classification is as follows:

- Stage I The carcinoma is confined to the corpus,
- Stage II The carcinoma has involved the corpus and the cervix,
- Stage III The carcinoma has extended outside the uterus but not outside the true pelvis.

Stage IV The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum.

According to this classification our material included 158 cases (75.6%) in stage I, 32 cases (15.8%) in stage II, 7 cases (3.3%) in stage III, and 11 cases (5.3%) in stage IV.

Management. Since 1963 the department has carried out a clinical experiment whereby all cases of endometrial carcinoma irrespective of the clinical stage, have been treated with primary radiation. Intracavitary and vaginal radium was the initial treatment selected in most cases (200) and external radiotherapy in the remainder.

Evaluation of the patient's general state of health was made on admission and the presence of associated conditions such as obesity, hypertension, diabetes or cardiovascular disease was noted. This was followed by a pelvic examination.

Table 1

Irradiators used in treatment of carcinoma of the uterine corpus

Length mm	Diameter mm		Wall thickness mm steel	Radium tube	Total filtration mm Pb	Dose rate at 1.5 cm
	Inner	Outer				
Normally sealed irradiators (% 0)						
0	3	6	1.5	Radium content 10 mg External length 17 mm Active " 11 mm Diameter 2.9 mm Filtration 2 mm Pb	2.4	32.2 rad/h = 100%
Addition of jackets						
2.5	6.1	8	1	No 1	2.6	30.8 rad/h = 96%
2.5	6.1	10	2	No 2	2.7	29.9 rad/h = 93%

under anesthesia and cervical dilatation and fractional curettage in order to determine uterine size and the depth and extent of tumor involvement. The procedure was carried out by a senior staff member with both gynecologic and radiotherapeutic qualifications. After clinical staging a preliminary plan of management was drawn up. Treatment was usually instituted forthwith as most of the patients had previously undergone curettage at local hospitals. In a few cases the uterine neoplasm was sometimes found to be secondary to carcinoma of the cervix or the ovaries and these cases have of course been excluded.

The cases in stage I and stage II were treated with intracavitary radium. The Heyman packing methods as modified by KJELGREN & JOHANSSON (11) was used in 187 cases (98.4%). The uterus was packed on three separate occasions fortnightly; it was sometimes necessary to insert a tandem at the third application on account of uterine shrinkage. The uterus was so atrophic in three cases (1.6%) that adequate packing could not be carried out and a tandem was inserted on each occasion.

Capsules containing 10 mg of radium were used for packing. The details of size and filtration are given in Fig. 3 and Table 1. The number and size of the capsules determined the duration of irradiation in each individual case (Table 2). The estimated delivered radiation dose at a distance of 1.5 cm from the nearest radium source was 3 000 rad.

The packing technique is simple. The idea is to fill the cavity with radium and if possible to distend and thin out the walls, thus shortening the distance

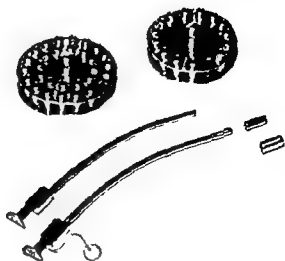


Fig 3 Instrumentarium used for intrauterine packing. Two jacket holders are seen uppermost, two applicators, of which the lower one is loaded with an irradiator with thread and tag are seen below and to the right are two irridators of different size.

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Table 3

Type of treatment in carcinoma of the uterine corpus stage I to II

	Cases treated	
	Number	Per cent
Intracavitary radium eventually followed by external radiation	200	91.7
External radiation only	8	3.7
Primary surgery	10	4.6
Total	218	100

Table 4

Method of judging the effect of primary radiotherapy in carcinoma of the uterine corpus stage I and stage II

	Number of cases		Total	Per cent
	Stage I	Stage II		
Total hysterectomy \pm bilateral salpingo-oophorectomy	99	20	119	63
Control curettage	59	11	70	37
Total	158	31*	189	100

One additional patient died of intercurrent disease one week before control curettage was planned.

Of the 208 cases in all stages treated with primary radiotherapy 200 received intracavitary radium (Table 3).

Six weeks after the completion of the radium treatment, total hysterectomy and bilateral salpingo-oophorectomy was performed in all suitable cases with stage I or stage II carcinoma. In the poor surgical risk cases where operation was considered inadvisable because of e.g. old age, hypertensive cardiovascular disease or diabetes, dilatation and curettage were carried out three months after radiation (Table 4).

Primary surgery was performed in only ten cases of the total material (Table 3). These operations were performed at local hospitals as a result of the incorrect interpretation of the clinical or histologic findings and the cases were later referred to the department for further care.

Table 2

Treatment times in hours required to deliver 1 000 rad at 1.5 cm depth with different numbers and sizes of irradiators in the treatment of carcinoma of the uterine corpus

Number of irradiators	Irradiator size		
	0	1	2
6	15 1/2	19	21 1/2
7	14 3/4	18	20
8	14	17	18 3/4
9	13	16 1/4	17 1/2
10	12 1/4	15 1/4	16 1/2
11	11 1/2	14 1/2	15 1/2
12	10 3/4	13 3/4	14 3/4
13	10 1/4	13 1/4	14
14	9 3/4	12 3/4	13 1/2
15	9 1/4	12 1/4	13

between the radium and the outermost layers of the uterus. Each capsule has a thread attached, which is tagged and numbered for easy removal. The cavity is packed in rows from the fundus downwards, the length of thread lying outside the vagina and held in the hand, indicating the position of the capsules. The cavity is packed as tightly as possible to accomplish which Hegar's dilators may be carefully burrowed between the capsules lying in the uterus to make room for more. The normal uterus is not readily distensible, but a uterus which is the site of a carcinoma can usually be ballooned out, thus ensuring satisfactory contact between the radium and the tumor. In experienced hands, perforations are uncommon and, as will be shown later, rarely troublesome. One or two capsules are usually placed in the cervix. Should the uterine cavity be very large the capsules may be enlarged by the addition of jackets (Fig. 3 and Tables 1 and 2). Bladder and rectal dosimetry is carried out after each application. Roentgen examination is performed if perforation is suspected. If the capsules are found to be well clustered this damage is improbable.

Vaginal radium was inserted at the first two packings in order to diminish the risk of vaginal metastases. The estimated radiation dose delivered to the vault was 3 000 rad at a depth of 1 cm. Following radium, cases in stage II were usually given supplementary external radiotherapy with ^{60}Co or 34 MeV betatron units 4 000 rad being delivered to the parametria and the pelvic walls. Cases with advanced disease or large tumor volumes in stages I and II were treated preliminarily with external radiotherapy followed by intracavitary radium.

tensive disease contributed to this decision. In the remaining cases the uterus could be packed despite the perforation. There were no cervical tears.

Laceration of the vaginal mucosa attributable to excessive vaginal packing with gauze occurred in two cases (1%). In one of these, the laceration had to be stitched.

Hemorrhage during packing occurred in two cases (1%). In one of these there was associated perforation of the uterus and a small blood transfusion (400 ml) was given.

Fever The presence of radium in the uterus often resulted in a slight rise in body temperature but this did not appear to have any clinical significance. Only ten cases (5%) ran a temperature of 38°C or more for two or more days. Five were in stage I, three in stage II and two in stage IV. The fever responded satisfactorily to antibiotic. Management was otherwise unaffected. None of the patients developed salpingitis. However, all those with a past history of salpingitis were given antibiotics for a week as a prophylactic measure.

Radiation necrosis of the vaginal vault This complication was noted in two cases (1%). Intracavitary radium had been supplemented by external radiotherapy in both and the lesions made their appearance after subsequent hysterectomy. They caused little or no trouble and healed spontaneously.

Deep venous thrombosis of the leg developed in one case a few days after the second radium application. The patient was 70 years old and had had recurring promelia prior to treatment. In addition she had carcinoma of the breast and skin. The case was treated with anticoagulants but was otherwise managed according to plan.

Late bladder and rectal complications The analysis is of course incomplete as the observation time is short. Five cases (2.5%) complained of bladder trouble one to three years after treatment with cystoscopic evidence of trigonal oedema. In two of these necrotic areas in the bladder mucosa were revealed. The symptoms were in all instances temporary and responded to simple treatment with urinary antiseptics. In four cases (2%) there were late symptoms from the sigmoid colon or the rectum. These were mild and of short duration and no ulcerated areas were seen at sigmoidoscopy.

There were no deaths attributable to packing.

Discussion

Several authors (1, 3, 18, 21, 25) have reported the incidence of residual carcinoma after primary radiation for endometrial carcinoma. The superiority

Results

The histologic studies were carried out jointly by the senior staffs of the departments of Pathology and Gynecological Radiotherapy. The primary cure rate in the 189 cases with stage I or stage II endometrial carcinoma treated with primary radiation was 174 cases (92.3%). The 5 year recovery rates are obviously not yet available.

The primary cure rate in stage I was 149 cases out of 158 (94.5%). The histologic specimens in 87% of the cases presented no traces of malignancy, a hyaline membrane replacing the endometrium. A pitheum without signs of vitality was present in 8% of these cases, and residual viable carcinoma in 5%. Further analysis revealed that the primary cure rate in the 99 cases in which supplementary hysterectomy was performed was 91%. No residual malignancy was present in the specimens obtained from curettage.

The primary cure rate in cases in stage II of endometrial carcinoma was 25 out of 31 (80.8%). Death of intercurrent disease occurred in an additional case one week before control curettage was planned and the effect of radiation could not be judged.

Analysis of the complications arising from packing disclosed that these were few and far between and to all intents and purposes without clinical significance. The hospital records of the 200 cases of endometrial carcinoma in all stages receiving intracavitary radium were studied, and the following complications were noted. A 100% follow up was maintained.

Pyometra Six cases (3%) had pyometra at the time of admission. Packing was carried out as planned, and the condition cleared up spontaneously as treatment progressed. In six other cases (3%), pyometra developed in the course of the treatment, in two of these packing was delayed for a few days while the uterus was drained. Apart from this the management was unaffected by this complication.

Perforation of the uterus Diagnostic procedures carried out before the treatment began resulted in perforation of the uterus in twelve cases (6%). Seven of these occurred in local hospitals and five in our department. The uterus could be packed as planned in all cases but one in which a tandem was inserted at the first application. In nine other cases (4.5%) the uterus was perforated as a direct result of the packing. Light of these cases were in stage I the other in stage II. In one case, a tandem was substituted. In another case the perforation necessitated a reversal of the usual time sequence of therapy, and the patient was given external radiotherapy followed by packing, the fact that she had ex-

SUMMARY

The preliminary results of a three year investigation of an unselected material of all known cases of endometrial carcinoma in a specific region of the north of Sweden are presented. Cases in stage I and stage II were treated with intracavitary radium followed if possible by hysterectomy and bilateral salpingo-oophorectomy. Curettage was performed three months after radiotherapy in the remainder. The primary cure rate was 95 % in the stage I cases and 81 % in the stage II cases.

ZUSAMMENFASSUNG

Es wird über die vorläufigen Resultate einer dreijährigen Untersuchung sämtlicher behandelten Fälle von Endometrialcarcinom in einem gewissen Bezirk in Nord Schweden berichtet. Fälle im ersten und zweiten Stadium der Erkrankung wurden intracavitär mit Radium behandelt und wo möglich erfolgte anschließend Hysterektomie und beiderseitige Entfernung der Tuben und Ovarien. Curettage wurde in den anderen Fällen drei Monate nach der Bestrahlung ausgeführt. Die primäre Heilungsrate war 95 % der Fälle im Stadium I und 81 % im Stadium II.

RÉSUMÉ

Les auteurs présentent les résultats préliminaires d'une étude ayant duré trois ans dans tous les cas non sélectionnés de cancers de l'endometre dans une région déterminée du nord de la Suède. Les cas aux stades I et II ont été traités par le radium intracavitaire suivi si possible d'une hystérectomie et d'une salpingo oophorectomie bilatérale. Un curetage a été fait dans les autres cas trois mois après la radiothérapie. Le taux de guérison primaire a été de 95 % dans les cas du stade I et de 81 % au stade II.

REFERENCES

1. ARNOLD A., STANBRO W. and VOLAV J. F. The use of multiple sources of radium within the uterus in the treatment of endometrial cancer. *Amer. J. Obstet. Gynec.* 55 (1948) 64.
2. CANDIS D. J. Cervical treatment of patients with corporal carcinoma. In: *Carcinoma of the uterine cervix, endometrium and ovary* p. 257. Year Book Medical Publishers Chicago 1962.
3. CHAIK P. M. Technique and evaluation of pre-operative radium therapy in adenocarcinoma of the uterine corpus. In: *Carcinoma of the uterine cervix, endometrium and ovary* p. 235. Year Book Medical Publishers Chicago 1967.
4. CORSEDAEN J. A. *Gynecologic cancer*. Third edition. Williams & Wilkins Baltimore 1967.
5. FURUHJELM M. Surgical treatment of carcinoma corporis uteri. *Acta obstet. gynec. scand.* 36 (1967) 470.
6. GRAHAM J. B. Treatment of choice in cancer of the uterine corpus. *New Engl. J. Med.* 254 (1956) 1117.
7. HENMAN J. The Radium's mmet experience in postoperative radiological treatment of cancer of the corpus uteri. *Acta radiol.* 18 (1937) 93.

of the packing over the tandem technique has been well documented (1, 18) ARNFSÖN (1), for example, stated that the proportion of cases of residual malignancy dropped from 77 to 26 % on changing from the tandem to the packing technique. The present results, a primary cure rate of 95 % in an unselected material with endometrial stage I carcinoma, are much better than any hitherto published. It should be borne in mind that the material is heterogeneous with large variations in the differentiation and extension of the growth as well as in uterine size.

Sceptics may query the validity of the histologic studies, both in respect of the number of histologic sections examined and the criteria by which cell vitality has been assessed. We are fully aware that even careful curettage may fail to reveal malignancy lurking deep in the myometrium. Although these considerations may somewhat limit the value of the study, it is hoped that it may provide food for thought. Ever since REYNOLD'S contribution in 1922, the impression has prevailed in many quarters that endometrial carcinoma, being derived from adenomatous tissue, cannot be cured by irradiation. It cannot be disputed that the intrauterine application of multiple sources delivers a lethal radiation dose to most endometrial carcinomas. The application must, however, be carried out properly in order to ensure the delivery of a uniform dose to the entire tumor. Of equal importance is adequate dosage, since a very high irradiation dose is necessary to cure this relatively radioresistent growth. Fortunately, the extreme radioresistance of the uterine walls makes the intracavitary application of such doses feasible.

We do not advocate radiotherapy alone as the treatment of choice for endometrial carcinoma. The fact that failure is occasionally encountered makes supplementary surgery mandatory in the good risk case. Cases with early carcinoma of the highly differentiated type may very well be treated with primary surgery. Their selection is not always so easy, however, and error of judgment may lead to serious consequences. We therefore believe that the standard treatment of all cases of endometrial carcinoma should be primary radiation, supplemented where possible by hysterectomy and bilateral salpingo oophorectomy. The radiation induced uterine shrinkage makes operation easier, and the above mentioned cancerocidal effect diminishes the risk of squeezing tumor cells into surrounding lymphatic and vascular channels at operation. Even if radiation fails to eradicate the growth, there is experimental evidence that irradiated malignant cells dispersed at the time of surgery are less liable to implantation elsewhere. Finally, the adoption of preliminary radiotherapy as a standard measure ensures that all cases of endometrial carcinoma are referred to a therapeutic centre. It is reasonable to suppose that centralization must lead to a degree of diagnostic and therapeutic expertise that cannot easily be obtained in any other way.

MODIFICATION OF EFFECTS OF PROTON AND GAMMA RADIATION ON THE RECTAL MUCOUS MEMBRANE BY LOCAL HYPOXIA

by

STIG STENSON

The influence of oxygen on the radiosensitivity of living cells is well known (GRAY *et coll* 1953 GRAY 1957 FOWLER 1966 RUBIN & CASARETT 1968). It has been shown in a variety of organisms that the anoxic cell is 2 to 3 times less sensitive to radiation of low linear energy transfer (LET₀₀) than the normally oxygenated cell (ALPER & HOWARD-FLANDERS 1956 DESCHNER & GRAY 1959). The idea to use this phenomenon for protection of healthy tissues when malignant tumours are treated by radiotherapy has been advanced by CHURCHILL DAVIDSON *et coll* (1960) and STECKEL *et coll* (1967).

In radiotherapy of carcinoma in the pelvis the main critical organ in consideration of dose is the rectum (TODD 1938 INGELMAN SUNDBERG 1947 KOTTMEIER & GRAY 1961). In a recent study (STENSON 1969a) high energy protons and roentgen radiation were found to induce the same type of damage to the rectal mucous membrane but there was a difference in relative biologic efficiency

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- II — and BENNER S. Further experience with radiotherapy in cancer of the corpus of the uterus. *Acta radiol* 27 (1946) 328
- 9 — REUTFRWALL O and BENNER S. The Radiumhemmet experience with radiotherapy in cancer of the corpus of the uterus. *Acta radiol* 22 (1941), 14
- 10 JAVERT C. T. and RENNING E. L. Endometrial cancer. Survey of 610 cases treated at woman's hospital (1919—1960). *Cancer* 16 (1963) 1057
- 11 HJELIGREN O and JONSSON L. Further development of the Heyman radium packing method. *Acta radiol Ther Phys Biol* 3 (1965) 143
- 12 KOTTNEIER H. L. Radiotherapy in uterine cancer. *Progr Gynec* II (1950) 574
- 13 — Carcinoma of the female genitalia. Williams & Wilkins Baltimore 1953
- 14 — Carcinoma of the corpus. Its classification and treatment. *Gynaecologia* 138 (1954) 287
- 15 — Klinische Fortschritte. In *Gynakologie* p. 419. Herausgegeben von T. Antoine Urban & Schwarzenberg Wien 1954
- 16 — Carcinoma of the corpus uteri. Diagnosis and therapy. *Amer J Obstet Gynec* 78 (1959) 1127
- 17 — The role of radiotherapy in gynecology. *Postgrad Med* 25 (1959) 124
- 18 LEMPE I. Combined surgical and radiological treatment for endometrial carcinoma. *Proc natl Cancer Conf* 1 (1952) 753
- 19 LINDGREN L. The prognosis of carcinoma of the endometrium in its different stages treated by surgery combined with post operative radiotherapy. *Acta obstet gynec scand* 36 (1957) 426
- 20 LONG R. T. L. SPRATT J. S. and SALA J. S. Problems in the management of advanced endometrial carcinoma. *Amer J Obstet Gynec* 87 (1953) 942
- 20 LONG R. T. L. SPRATT J. S. and SALA J. S. Problems in the management of advanced endometrial carcinoma. *Amer J Obstet Gynec* 87 (1963) 942
- 21 MARTIN C. L. and MARTIN J. A. Can endometrial carcinoma of the uterine fundus be eradicated with radium therapy? *Amer J Roentgenol* 89 (1963) 491
- 22 NOLAN J. F. Symposium on endometrial cancer. *Amer J Obstet Gynec* 81 (1961) 1099
- 23 NORDLANDER E. Operative treatment of carcinoma of the corpus uteri. *Acta obstet gynec scand* 32 (1953) 211
- 24 PRICE J. J. HAIN G. A. and ROMINGER C. J. Vaginal involvement in endometrial carcinoma. *Amer J Obstet Gynec* 91 (1965) 1060
- 25 RANDALL I. H. and GODDARD W. H. A study of 531 cases of endometrial carcinoma. *Surg Gynec Obstet* 103 (1956) 221
- 26 REGAUD C. Influence de la durée d'irradiation sur les effets déterminés dans le testicule par le radon. *C. R. Soc Biol (Paris)* 86 (1922) 787
- 27 RINGERTZ N. SJOSTROM A. TORNERBERG O. and SWENSON D. (Editors). *Cancer incidence in Sweden 1958—1961. The Cancer Registry. The National Board of Health* Stockholm 1960 1962 1963 and 1965
- 28 RUBIN P. GERLE R. D. QUICK R. and GREENLAW R. H. Significance of vaginal recurrence in endometrial carcinoma. *Amer J Roentgenol* 89 (1963) 91
- 29 SHERMAN A. J. *Cancer of the female reproductive organs*. C. V Mosby, St Louis 1963
- 30 WAY S. *Malignant disease of the female genital tract*. J. & A. Churchill London 1951

Table 1

Semi quantitative comparison of changes in deoxygenated and normally oxygenated segments of rat rectum in groups I—II of proton and gamma irradiated rats

Rectal treatment	Dose rad	Hours after irradiation							
		40—60		60—80		80—100		100—120	
		a	b	a	b	a	b	a	b
<i>Group I proton irradiated (13 rats)</i>									
Nor-epine	1 400			±	++	—	±	+++	+++
phrine and	2 000			±	++++	±	+++	+	+++
sodium sulfite				0	+	+	+++	++++	+++++
				+	++	+	++		
	2 400			+	±	+	+++		
				+	++	+	±		
	2 800			+	+	+	±		
				+	+				
<i>Group II gamma irradiated (24 rats)</i>									
Nor-epine	1 500					++	±	+	±
phrine and						+	++	0	±
sodium sulfite						+	++	0	±
								+	+
	2 000			+	++			0	±
				±	+	++	+++		
						+	±		
						++	+++		
						+	±		
	2 500	0	+	+	+++	++	±		
		0	+	+	+++	+	++		
		0	+	+	++	++	+++		

a = specimen from the deoxygenated part of the rectum b = specimen from the normally oxygenated part of the rectum 0 to +++ signify different grades of radiation induced changes as defined in a preceding study (Srinivasan 1969a)

those animals that were predetermined to survive more than 90 hours. The source-skin distance was 40 cm, the beam axis being perpendicular to the skin (Fig. 1). The dose rate was 280 rad/min as determined by a miniature ionization chamber in the rectum. The homogeneity of the beam at the rectal level was $\pm 5\%$ (Cedergren 1969). The doses were 1 500 to 2 500 rad.



Fig 1 Lateral roentgenogram of rat showing the position at gamma irradiation beam perpendicular to skin *a*=section of rectum giving specimen *a* *b*=section of rectum giving specimen *b*

The possibility of protecting the rectal mucous membrane from radiation damage by creating a state of local hypoxia during irradiation through rectal injection of solutions with vasoconstricting and oxygen reducing substances was demonstrated preliminarily by LARSSON & STENSON (1965). The present paper aims at a closer evaluation of this technique.

Material and Methods

Seventy seven inbred Sprague-Dawley female rats weighing 180 to 250 g were used in the study.

Proton irradiation Twenty eight rats (group I) were irradiated with a well collimated 187 MeV proton beam (cf LARSSON 1961). The beam was used without intervening absorbers. The dose rate was 35 to 125 rad/min and the mean LET ∞ at the rectal level 0.5 keV/ μ m. The doses were 1400 to 2800 rad and the fluence homogeneity in the rectal area was in the range $\pm 5\%$, determined as described by FALKNER et al (1959). The rats which should live for less than 90 hours had the whole abdominal cavity uniformly irradiated. Those which should live for 90 to 120 hours had, at irradiation, a zone of about 2 cm of the upper part of the abdominal cavity protected. This protection permitted the animals to survive the desired period (cf STENSON 1969b). During the irradiation, the position of the animals was checked by means of a television arrangement.

Gamma irradiation Forty four rats (groups II—VI) were irradiated with a 3000 Ci ^{60}Co source (Siemens Gammatron III). The abdominal cavity of the rats was irradiated uniformly except for a 2 cm zone of the upper abdomen of

minutes before irradiation and was continued until the end of irradiation. Finally the abdominal incision was re-opened, the ligature and the catheter removed and the abdominal wall closed.

The oxygen reducing treatment in group I (proton irradiated rats) and group II (gamma irradiated rats) started with the infusion of 1 ml norepinephrine and continued after one minute with sodium sulfite solution.

The rats in group III received only physiologic saline as rectal treatment, group IV norepinephrine and saline, group V only 0.1 mol Na₂SO₃ and group VI no rectal treatment at all during irradiation after operation. Finally five rats (group VII) received the same rectal treatment as groups I and II but were not irradiated. The rectal treatment, the type of irradiation and the doses given to the different groups are presented in Tables 1 and 2.

The observation periods were 40–60, 60–80, 80–100 and 100–120 hours.

Histologic technique. At the end of the observation periods, the rats were sacrificed by a blow on the head and histologic specimens were taken from that part of the rectum which had been irradiated whilst also having chemical treatment, i.e. anally to the ligature and thus easily recognized (specimen a). Another rectal specimen (specimen b) was taken orally to the ligature, i.e. in irradiated but normally oxygenated region.

The same histologic technique for preparation of the specimens was used as in a preceding study (STENSON 1969a) and the criteria for the semi-quantitative histologic grading 0 to V+ were also the same. The specimens were about 1 cm in length and sectioned at approximately 7 μ m forming a complete series at planes less than 0.5 mm apart. The sections were assessed as unknown independently several times by two investigators. When the lesions were unevenly distributed in various parts of the intestinal mucosa the final grading was the highest one observed. In the 512 assessments of the 128 specimens the variations between different gradings were small on different occasions and between the two investigators. Thus the differences were higher than one degree only in one specimen where the grading ranged from II to II+. Divergencies in the gradings essentially concerned grades 0 to I+ and I+ to II+.

Measurement of oxygen tension. The effect of norepinephrine and sodium sulfite on the oxygen tension in the rectum was studied separately in four rats. A modified Clark platinum pO₂-electrode (Beckman International Instrument S.A.) connected for amplifying to a Beckman Physiological Gas Analyzer Model 160 was used (cf. GRANJO & ULFENDAH 1962).

The anaesthetized rat was opened with an abdominal incision and put dorsally on an inclined wooden platform with the head uppermost. One millimeter norepi-

Table 2

Semi quantitative comparison of changes in specimen a and specimen b of the rat rectum treated with different solutions with and without irradiation of groups III to VII

Experimental groups	Rectal treatment	Dose rad	Hours after irradiation (or treatment)								
			40—60		60—80		80—100		100—120		
			a	b	a	b	a	b	a	b	
<i>Gamma irradiated</i>											
III 4 rats	0.9 % NaCl	2 000	+	+	++	++	++++	+	++++	++++	++++
IV 4 rats	Nor ep 0.9 % NaCl	2 000	0	++	+	++	+		++	++++	++++
V 4 rats	Sodium sulfite	2 000	+	+	+	++	++		+++	+++	++++
VI 4 rats	0	2 000	++	++	++	+	+++		+++	+++	++
VII 5 rats	Nor epinephrine and sodium sulfite	0	0	0	0	0	0	0	0	0	0

a = specimen a b = specimen b 0 to ++ signify different grades of radiation induced changes as defined in a preceding study (Stenson 1969a)

During the irradiation the position of the animals was checked by means of a television arrangement

Oxygen reducing treatment The solutions, which were intended to create a state of local hypoxia in the rectal mucous membrane were norepinephrine (norepinephrine 1 mg/ml Astra Sweden), and 0.1 mol sodium sulfite solution in deionized water, i.e. a slightly hypotonic solution with a pH of approximately 9. They were used in combination or each separately.

Before treatment all the rats were anaesthetized with mebumal sodium (nembutal, Abbott), 4 mg/100 g body weight, given intraperitoneally. A thin rubber catheter, perforated 1 cm from its closed tip, was introduced through the anus 3 to 4 cm into the rectum. The abdomen was opened with an abdominal incision and the catheter was fixed in the rectum by a silk ligature carefully put around the rectum. The abdominal incision was then closed and the animal put dorsally, for treatment, on an inclined wooden platform with the pelvis uppermost (Fig 1). This position permitted filling of the rectum between the anus and the ligature with the test solution. The rectal treatment was given as a drop infusion 2.7 ± 0.5 ml/min at a pressure of 80 cm H₂O. The infusion started 5 to 6



Fig. 3 Photomicrographs of rectal mucous membrane of rat 84 hours after irradiation with 2500 rad of gamma rays *Left*) grade II + damage of the deoxygenated part of rectum *Right*) grade III + damage of the normally oxygenated part of rectum HFX — coun $\times 100$

was. One rat was excluded from the study because of the leakage of the rectal treatment to the rectum orally to the ligature.

Immediately after irradiation three rats in groups III—IV died from the anaesthetic but these animals were at once replaced with others to make these small groups complete. All the rats which died during the planned observation period were excluded from the investigation because of post mortem changes in the rectal mucous membrane.

At post mortem examination specimen *a* (chemically treated) and specimen *b* (normally oxygenated) were easily identified. There were small haemorrhages in the rectal wall and an opening in the rectal mesentery indicating the place of the ligature at irradiation (Fig. 1).

Macro- and microscopic examinations. Macroscopically, in the entire material there was no obvious damage and no difference between specimens *a* and *b* or between the effects of proton and gamma irradiation.

At the microscopic examination no qualitative differences were observed



Fig 2 Photomicrographs of rectal mucous membrane of rat 79 hours after irradiation with 2000 rad of protons *Left* grade I + damage of the deoxygenated part of rectum *Right* grade III + damage of the normally oxygenated part of rectum HTX — eosin $\times 100$

nephrene was infused into the rectum and, after a few minutes, the platinum electrode was put into the rectal lumen. When the electrode potential was stable, 0.1 mol sodium sulfite was dropped on the outside of the rectum at the level of the electrode, the electrode potential being recorded every minute. Before and after each experiment the electrode was calibrated at 37° C with 0.1 mol sodium sulfite ($pO_2 = 0$ mm Hg) and air saturated water ($pO_2 = 155$ mm Hg).

Results

Nine of the twenty eight rats in group I died during the predetermined observation period. Four died from anaesthesia during the first hours after irradiation, one from operative complication and four on the 4th day after irradiation, obviously from radiation induced damage to the intestine. In group II, three of the twenty eight rats died from operative complications: two of them from bleeding into the peritoneal cavity and one from rupture of the abdominal

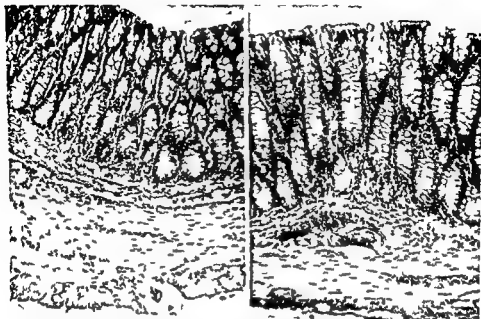


Fig 5 Photomicrographs of rectal mucous membrane of rat 90 hours after treatment with norepinephrine and sodium sulfite for 15 minutes without irradiation Grade 0 damage in both specimen *a* (left) and specimen *b* (right) HTE — eosin $\times 100$

degree of damage in the *a* and *b* specimens. In no gamma rat of this observation period was there a difference of 2 grades.

The two proton rats irradiated at 2 000 rad and one proton rat irradiated at 1 400 rad in the 100–120 hour period displayed a grade 2, a grade 1, and a grade 0 difference respectively, and the five rats gamma irradiated at 1 500 rad displayed differences of 1 or 2 grades.

In the rats of group III treated with saline during gamma irradiation there was no difference between the *a* and *b* specimens compared (Fig 4). One rat in group IV treated with norepinephrine and saline during gamma irradiation had no effect of the chemical treatment, and in the other rats of this group differences of 1 to 2 grades were noted. In group V, treated with 0.1 mol Na₂SO₃ during gamma irradiation, a grade 1 difference was noted at the 60–80, 80–100, and 100–120 hour periods. The rat sacrificed at the 40–60 hour period showed no effect of the chemical treatment. At 2 000 rad of gamma radiation, the rats of group VI operated upon but without rectal treatment had the same degree of damage in the *a* and *b* specimens. In the five rats of group VII receiving norepinephrine and sodium sulfite for 15 minutes without irradiation, there was no reaction in any specimen (Fig 5).

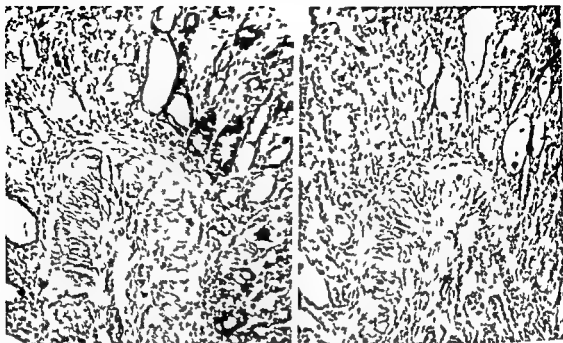


Fig 4 Photomicrographs of rectal mucous membrane of rat 90 hours after rectal treatment with physiological saline during irradiation with 2 000 rad of gamma rays Grade IV+ damage in both specimen *a* (left) and specimen *b* (right) HTA — eosin $\times 100$

between the effects of proton and gamma rays on the rectum. The results of the microscopic grading of the damage are given for groups I—II in Table 1 and for groups III—VII in Table 2. In 74 % of all proton irradiated rats of group I (14 out of 19) the changes in specimen *a* were one or two grades lower than in specimen *b*, and in 26 % the grading was the same in the specimens compared. The corresponding figures for the gamma irradiated rats of group II were 83 % (20 out of 24) and 17 %, respectively. In no assessment was the grade of damage in specimen *a* higher than in that of specimen *b*.

In the 40–60 hour period, the three rats gamma irradiated at 2 500 rad all displayed a grade 1 difference in the specimens compared.

The 60–80 hour period contained thirteen proton or gamma irradiated rats. In nine of them there was a grade 1 difference in three no difference and in one animal (2 000 rad protons) there was a difference of 3 grades between the *a* and *b* specimens compared.

Eight proton and eleven gamma irradiated rats were sacrificed 80–100 hours after the irradiation. Three proton rats (2 000 rad) displayed a difference of 2 grades (Fig 2), three a grade 1 difference and two no difference between the specimens compared. Eight of the eleven gamma rats showed a difference of 1 grade between the specimens compared (Fig 3), and three had the same

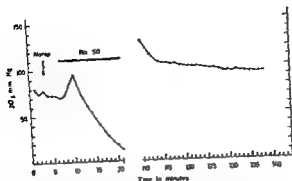


Fig 6 Rat No 1 Diagram showing the changes in oxygen tension in the rectal lumen of rat during treatment with norepinephrine and 0.1 mol sodium sulfite solution

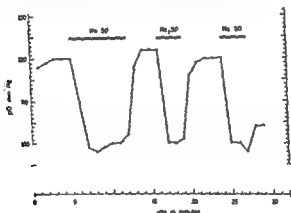


Fig 7 Rat No 3 Diagram showing the changes in oxygen tension in the rectal lumen of rat treated discontinuously with 0.1 mol sodium sulfite solution on the outside of the rectum

In preceding studies (FALKNER et coll 1959 STEVSON 1969a) comparison was made between the effects of high energy protons and roentgen rays on rabbit skin and the rectum of the rat. In the present study a comparison was made between the effects of protons and gamma rays. A dose of 2000 rad of protons creates approximately the same histologic effects on the rectal mucous membrane as 2000 rad of gamma rays (cf Table 1).

The distension of the rectum by filling it with physiologic saline (group III) or the application of a ligature around the rectum containing a catheter (group VI) did not influence the radiosensitivity of the mucous membrane. Further

Changes in rectal oxygen tension The following observations were made in four rats subjected to a drop of sodium sulfite solution on the outside of the rectum through an abdominal incision.

Rat No. 1 had a decrease in oxygen tension from 70 to 80 mm Hg to about 15 mm Hg in 14 minutes after the onset of the sulfite drop. The drop was then stopped and the abdominal incision closed. After 1 1/2 hour, when the rat was still under anesthesia, the oxygen tension in the rectum was again measured. It soon became stable at 90 to 100 mm Hg (Fig. 6). The rat survived the experiment.

Rat No. 2 reacted similarly but the decrease in oxygen tension was slower than in rat No. 1 and this rat died about half an hour after closing of the abdominal incision.

In order to examine the possibility of reproducing changes in the oxygen tension in the rectum during the experiment, rats Nos. 3 and 4 were subjected to a discontinuous drop of sodium sulfite to the rectum. As seen from the curves in Fig. 7, the decrease in oxygen tension followed immediately upon the start of the drop, and at the withdrawal the oxygen tension was restored. The procedure was repeated twice with the same result. A similar result was obtained in rat No. 4. In both the latter rats the respiration and the intestinal circulation was seemingly undisturbed during the oxygen measurement.

Discussion

The time required for complete renewal of the cell population of the rectal mucosa in the rat has been estimated to about 6 days (BERTALANFFY 1960). After roentgen irradiation of the abdominal cavity with 1 000 rad or more the rat will suffer acute intestinal radiation death within 4 days (QUASTLER et coll. 1951). Since the same holds for proton irradiation (STENSON 1969b), the acute intestinal radiation death makes a long term study impossible with the radiation technique used in the present study, as histologic damage cannot be demonstrated in the rectum of rat after 1 000 rad or less (STENSON 1969a). Fortunately, however, the important period after irradiation i.e. when the changes become serious in connection with the intestinal radiation death, can be studied at 80–100 hours. The radiation doses used in the investigation created mucosal changes which could easily be graded histologically.

The results indicated that the experimental conditions used permit a study of the radioprotective effects of norepinephrine and sodium sulfite, and it was possible to compare specimens from deoxygenated and normally oxygenated segments of the rectum in each single animal. So the possible influence of the individual radiosensitivity was eliminated.

RÉSUMÉ

L'injection rectale de noradrénaline et de sulfite de sodium pendant l'irradiation de l'abdomen du rat par les rayons gamma du cobalt ou par des protons de haute énergie réduit en partie les graves lésions histologiques constatées sur la muqueuse rectale après son irradiation. L'effet protecteur est attribué à l'état hypoxique de la paroi rectale du à ce traitement.

REFERENCES

- ALPER T and HOWARD-FLANDERS P The role of oxygen in modifying the radiosensitivity of *E. coli* B. *Nature* 178 (1956) 978
- BERTALANFFY F D Mitotic rates and renewal times of the digestive tract epithelia in the rat. *Acta anat* 40 (1960) 130
- CEDERLUND J Personal communication (1969)
- CHURCHILL-DAVIDSON I Personal communication. Cited by P Howard Flanders and O C. L. Scott in *Radiology* 74 (1960) 946
- DESCHNER E C and GRAY L H The influence of oxygen tension on X ray induced chromosomal damage in Ehrlich ascites tumour cells irradiated in vitro and in vivo. *Radiat Res* 11 (1959) 115
- FALKNER B, LARSON B and STÉNOV S Effects of single dose proton irradiation of normal skin and $\lambda\lambda_2$ carcinoma in rabbit ears. A comparative investigation with protons and roentgen rays. *Acta radiol* 52 (1959) 217
- FOWLER J Radiation biology as applied to radiotherapy. In *Current topics in radiation research* Volume 11 p 305. Edited by M Fbert and A Howard. North Holland Amsterdam 1966
- GRANQVIST G and ULFENDAM H R. Factors influencing the properties of electrodes for the continuous measurements of oxygen tension in tissues. *Acta Soc Med Upsalien* 67 (1962) 107
- GRAY L H Oxygenation in radiotherapy. *Brit J Radiol* 30 (1957) 403
- COOPER A D, EBERT M et coll. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Brit J Radiol* 26 (1953) 638
- NOFELMAN SUNDBERG A Rectal injuries following radium treatment of cancer of the cervix uteri. *Acta radiol* (1947) Suppl No 64
- HOTTINGER H L and GRAY M J Rectal and bladder injuries in relation to radiation dosage in carcinoma of the cervix. *Amer J Obstet Gynec* 82 (1961) 74
- LARSON B Pre therapeutic physical experiments with high energy protons. *Brit J Radiol* 34 (1961) 143
- and STÉNOV S Reduction of radiation damage to the intestinal mucous membrane by local hypoxia. *Nature* 203 (1965) 364
- QUATLER H, LANZ E, P KELLER M E and OSBORNE J W Acute intestinal radiation death. *Amer J Physiol* 164 (1963) 546
- ROBIN P and CASARETT G W The radiopathologic basis for oxygen breathing in radiotherapy. In *Clinical radiation pathology* Vol 2 p 934. Saunders Company Philadelphia 1968
- STICKEL R J, MACLOWRY J D, HOLLAND J M et coll. The Seldinger catheter in radiation therapy. *Radiology* 89 (1967) 329

norepinephrine and sodium sulfite did not seem to damage the rectal mucosa (group VII)

The results in groups I and II confirm that norepinephrine and sodium sulfite protect the rectal mucous membrane, as previously demonstrated by LARSSON & STENSON 1965. Both norepinephrine and sodium sulfite seemed to contribute to this effect (cf groups IV and V). The rectal treatment in group I seemed to be slightly more effective than that in group II after the same dose, 2 000 rad. This may be due to the lower dose rate at proton irradiation giving approximately a 5 times longer treatment period than at gamma irradiation. The oxygen reducing treatment was thus applied longer in group I. After irradiation at 2 400, 2 500 and 2 800 rad, the difference between compared specimens was less (0 to 1 grade). The doses were apparently too high to permit the same protection as at 1 500 and 2 000 rad.

Determination of the oxygen tension could not be made in the thin rectal wall under the experimental conditions of the main experiment. By the supplementary technique introduced it was possible to show, however, that norepinephrine and sodium sulfite applied on the outside of the rectal wall, decreased the oxygen tension in the rectal lumen. Although proof is still lacking, it may be assumed that the oxygen reduction in the mucous membrane should have been at least as effective when the solution was applied in the rectal lumen, thus directly on the mucosa as when applied on the outside of the rectal wall.

The local radioprotective effect of the chemical treatment applied seemed to be due to the hypoxic state of the tissues during irradiation and seemed to be effective for both high energy protons and gamma irradiation. A practical consequence of this result might be that rectal damage can be reduced in patients irradiated therapeutically with radiation of low linear energy transfer.

SUMMARY

Rectal injection of norepinephrine and sodium sulfite during irradiation of the abdomen of the rat with cobalt gamma rays or high energy protons was found to partially reduce the severe histologic damage of the rectal mucous membrane that is usually seen after irradiation of this structure. The protective effect was ascribed to the hypoxic state in the rectal wall induced by the treatment.

ZUSAMMENFASSUNG

Bei Bestrahlung vom Bruch der Ratten mit Gammastrahlen und hochenergetischen Protonen wurde Norepinephrin und Natriumsulfid rectal eingespritzt. Dabei wurde eine Reduktion der Beschädigung der rectalen Schleimhaut partiell erreicht, die sonst bei Bestrahlung dieser Struktur histologisch meistens nachgewiesen wird. Es wird angenommen, dass die Schutzwirkung eine Folge der Hypoxie der Rectalwand war, die bei der Behandlung hervorgerufen wurde.

TELEGRAPHIC THERAPY IN CARCINOMA OF THE HYPOPHARYNX WITH SPECIAL REFERENCE TO CYTOLOGICALLY VERIFIED METASTASES IN THE NECK

by

GÖRAN WERNER and INGVAR BÄRBY

Carcinoma of the hypopharynx is now rare in Sweden. According to the Cancer Register the average annual number of patients during the period 1959—1963 was 61 of which 32 were men and 29 women (RINGERTZ et coll 1962—1967) corresponding to a morbidity of 8.1 per million inhabitants per year. About a third of the patients reported during 1955—1965 were treated at Radiumhemmet, these being from the central and northern parts of the country.

Material. A total of 195 out of 218 patients referred to Radiumhemmet with carcinoma of the hypopharynx during the period 1955—1965 received treatment and there was an increasing tendency to use high-energy therapy for this group of tumours (Table 1). In 23 patients during the same period their generally poor condition or the spread and advance of the disease precluded any treatment.

A total of 83 of the 195 patients treated received ^{60}Co therapy. Nine of these

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- STENSON S (a) Effects of proton and roentgen radiation on the rectum of the rat *Acta radiol Ther Phys Biol* 8 (1969) 263
- (b) Weight change and mortality of rats after abdominal proton and roentgen irradiation. A comparative investigation *Acta radiol Ther Phys Biol* 8 (1969) 423
- FOOD T F Rectal ulceration following irradiation treatment of carcinoma of cervix uteri *Surg Gynec Obstet* 67 (1938) 617

TELEGAMMA THERAPY IN CARCINOMA OF THE HYPOPHARYNX WITH SPECIAL REFERENCE TO CYTOLOGICALLY VERIFIED METASTASES IN THE NECK

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Table 3

Patients with carcinoma of the hypopharynx grouped according to site of tumour and survival after ^{60}Co therapy

	Site of lesion					Total numbers of patients	Per cent
	Vallecula epiglottis aryepiglottic fold	Posterior cord area	Pyramidal fossa	Pharyngeal walls	Growth into surrounding organs		
Number of patients	11	5	25	7	18	83	100
1 year survival	16/28	2/5	12/25	3/7	9/18	47/83	51
2 year survival	9/28	2/5	10/25	2/7	7/18	30/83	36
3 year survival	7/27	2/5	4/22	1/7	4/18	18/79	23
More than 5 year survival	6/24	1/4	3/16	—	1/15	11/65	17

Of the eleven patients who survived 5 years or more nine had growths in the upper hypopharynx and two in the lower hypopharynx.

Table 4

Distribution according to location of tumour in upper or lower part of the hypopharynx

	Total number of patients	Women		Men	
		Number	Per cent	Number	Per cent
Carcinoma of the upper part of the hypopharynx	63	10	16	53	84
Carcinoma of the lower part of the hypopharynx	12	8	67	4	33
Tumours with extended growth in both the upper and the lower parts of the hypopharynx	8	2	25	6	75

Co therapy as it became possible to treat increasing numbers of patients with high-energy technique (Table 1). Sixty-four of these were men and nineteen, i.e. 23%, were women (Table 2). This distribution between the sexes is markedly different from that which existed during the period 1939–1947 when JACOBSSON (1951) reported the results of roentgen treatment i.e. in a total of 322 patients of whom 119 were men and 203 women.

The mean age on admission in the present series of patients was 61 years.

Table 1

Type of treatment given in the different periods

Year of admission	Treated with roentgen	Treated with ^{60}Co	Not accepted
1955	19	3	3
1956	14	5	3
1957	32	6	2
1958	7	11	—
1959	5 (7)	9	3
1960	13	6	5
1961	8 (10)	9	3
1962	3	13	2
1963	2	5	—
1964	5	7	—
1955—1965	108 (112)*	83	23

* Four patients were operated upon before roentgen therapy

Table 2

Distribution according to age and sex of the 83 patients who received ^{60}Co therapy — The mean age of the men was 61 and that of the women 59 years

Age in years	Men	Women
30—39	2	—
40—49	9	11
50—59	13	8
60—69	27	6
70—79	13	3
Total number 83	64 (77 %)	19 (23 %)

were also subjected to therapy with 15 MeV electrons or 180 kV roentgen treatment. Sixty five of the eighty three patients were treated with kilocurie techniques and eighteen with a short distance gamma beam, the so called decacurie technique. The remaining 112 patients received roentgen irradiation.

Some degree of selection can be assumed to have taken place, inasmuch as the eighty three patients who received high energy therapy were considered prognostically more favourable than those who had roentgen irradiation during the same period. This was certainly true in the eighteen patients who received decacurie therapy and subsequently to a lesser extent in the patients given

Table 3

Patients with carcinoma of the hypopharynx grouped according to site of tumour and survival after ^{60}Co therapy

	Site of lesion					Total numbers of patients	Per cent
	Vallecula epiglottic aryepiglottic fold	Post cricoid area	Pyriform fossa	Pharyngeal walls	Growth into surrounding organs		
Number of patients	28	5	25	7	18	83	100
1 year survival	16/28	2/5	12/25	3/7	9/18	42/83	51
2 year survival	9/28	2/5	10/25	2/7	7/18	30/83	36
3 year survival	7/27	2/5	4/22	1/7	4/18	18/79	23
More than 5 year survival	6/24	1/4	3/16	—	1/15	11/65	17

Of the eleven patients who survived 5 years or more nine had growths in the upper hypopharynx and two in the lower hypopharynx.

Table 4

Distribution according to location of tumour in upper or lower part of the hypopharynx

	Total number of patients	Women		Men	
		Number	Per cent	Number	Per cent
Carcinoma of the upper part of the hypopharynx	63	10	16	53	84
Carcinoma of the lower part of the hypopharynx	12	8	67	4	33
Tumours with extended growth in both the upper and the lower parts of the hypopharynx	8	2	25	6	75

^{60}Co therapy as it became possible to treat increasing numbers of patients with high-energy technique (Table 1). Sixty-four of these were men and nineteen, i.e. 23%, were women (Table 2). This distribution between the sexes is markedly different from that which existed during the period 1939–1947 when JACOBSSON (1951) reported the results of roentgen treatment i.e. in a total of 322 patients of whom 119 were men and 203 women.

The mean age on admission in the present series of patients was 61 years.

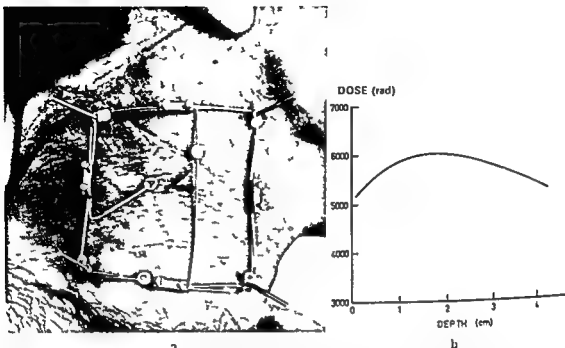


Fig 1 Technique for decacurie treatment a) A wire jig with four axial pins giving the directions of the central rays and the points for depth dose calculation b) Example of depth dose at point A on skin in (a) 4 000 rad was given to each one of the four fields

for the men and 59 years for the women. Only thirteen of the patients were under 50 and none was over 74 years old.

The series was divided into groups of patients according to the origin and location of the tumour (Table 3) and further subdivided into two groups, representing respectively carcinoma of the upper and lower parts of the hypopharynx (Table 4), drawing the borderline at the superior margin of the cricoid cartilage. Thus the first group includes carcinomas situated mainly in the external parts of the larynx and the piriform fossa; all the others referred to were carcinomas of the lower part of the hypopharynx, except eight patients in whom it was impossible to judge whether the tumour originated in the upper or lower parts of the hypopharynx. The reasons for this division were differences in the sex distribution and predisposing factors. Of the 83 patients treated, 63 had growths that had arisen in the upper part of the hypopharynx. The tumour had invaded adjacent organs such as the oesophagus or the base of the tongue in eighteen patients, the extent of the tumour in eight of these made it impossible to determine whether it had arisen from the upper or lower parts of the hypopharynx.

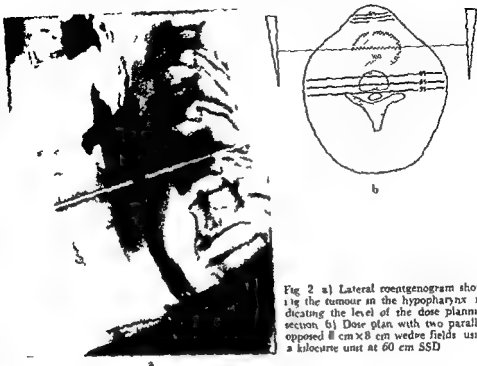


Fig 2 a) Lateral roentgenogram showing the tumour in the hypopharynx indicating the level of the dose planning section. b) Dose plan with two parallel opposed 8 cm x 8 cm wedge fields using a kilocurie unit at 60 cm SSD

All the neoplasms in this material were histopathologically verified as squamous-cell carcinomas mostly of a relatively poorly differentiated solid type.

Fifty two patients had demonstrable unilateral and nine patients had bilateral lymphatic gland metastases in the neck. The occurrence of these metastases was checked by cytologic investigation after aspiration biopsy (FRANZEN 1968) and additionally in five patients by extirpation of the gland and P.A.D. Multiple primary malignant tumours were present in seven patients; in three of these another primary malignant tumour had arisen prior to that in the hypopharynx and in four patients other primary malignant tumours occurred during the follow up period. Eighteen (22%) of the patients had had their first symptoms more than six months prior to admission to Radiumhemmet; in the others the period was less than six months.

Methods of examination. The size of the tumour was always determined by roentgen examination of the soft parts of the neck and contrast roentgen examination of the hypopharynx. Indirect laryngoscopy and hypopharyngoscopy

Table 5

Tumour dose and fractionation times in relation to 2 year survivals

Tumour dose in rad	Fractionation time in weeks					Total number	Per cent
	<5	5	6	7	>7		
<5 500	0/2	3/3		2/2		4/7	57
5 500—5 999	2/6	0/5	0/2	0/1		2/14	14.3
6 000—6 499	6/9	4/9	6/13	1/5	0/2	17/38	44.7
6 500—6 999	0/3	1/3	0/5	1/2	3/5	5/18	27.7
> 6 999	0/1		0/2	0/1	2/2	2/6	33
Total	8/21	7/20	6/22	4/11	5/9	30/83	36

were usually also used to determine the margins of the growth. These methods were combined with biopsy of the primary tumour. Thin needle aspiration biopsy (IRANZEN 1968) was always carried out when lymphatic gland metastases were possibly present in the neck. Direct extension of the growth into the regional lymphatic glands in the neck sometimes occurred when the primary tumour was advanced. It was then often impossible to determine whether or not the changes were due to lymphatic gland metastases. The doubtful cases were included among the lymphatic gland metastases.

Irradiation of these tumours was until 1955 carried out almost exclusively with conventional roentgen irradiation. Between 1955 and 1960 some patients with carcinoma of the hypopharynx were treated with ^{60}Co , employing the short distance gamma beam technique. Subsequent to 1960 most of the patients were irradiated with the kilocurie technique.

Short distance gamma beam technique (decacurie technique) Methods based on directing a number of circular radiation beams onto the tumour region from a radium source at a distance of 6 to 10 cm were developed. The technique was further developed when ^{60}Co sources with activities of 10 to 50 Ci (decacurie sources) for this type of therapy and more favourable dose distributions could be achieved (WAISTAM 1965). Dose calculations and duplication of the positions of the treatment fields were based on the production of individual wire jigs. These jigs were so designed that they followed the skin surface in the treatment area and could be fitted with axial pins for the central rays of the radiation beams and with indicators at the points selected for depth dose calculations (Fig 1 a).

The percentage contribution from each field was obtained for each of the

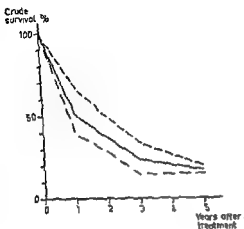


Fig. 3 Survival curves for the 83 patients with carcinoma of the hypopharynx who received ^{60}Co therapy. The total 5 year survival rate was 17% (—). The 5 year survival rates for 52 patients with cytologically verified lymph node metastases (---) and for 31 patients without verified lymph node metastases (- - -) were respectively 15% and 20%.

calculation points selected by rotating transparent flexible isodose diagram around the axial pins. Multiplication by the loading on each field and summation over all the fields gave the dose for each calculation point. The resultant depth doses for the points selected on the skin surface are illustrated by the depth dose diagram in Fig. 1b. The central rays of the beams were made to coincide with the directions of the axial pins of the wire jig and the entrance points were marked on the skin. By comparing the skin markings with the axis prior to each treatment it was possible to ensure that the patient maintained the correct position and that the skin markings did not change position, for instance due to reduction in the size of the tumour, an effect which could result in considerable overdoses in certain areas. The number of fields depended on the extent of the tumour. The treatment was in general 1 000 rad to one field a day. Each field was irradiated with about 4 000 rad to achieve a tumour dose of 6 000 rad. The average treatment time was 30 days.

The kilocurie technique. To determine the size of the tumour and the position of the spinal cord, a lateral roentgenogram was obtained for each patient in supine position and an enlargement indicator was inserted (Fig. 2a). The position of growths with considerable vertical extension was determined from two or more sections in each of which a strip of dental impression compound (Kerr) was cast to indicate the contour. A mask of the outline of the neck was made at the same time to duplicate the treatment position.

Dose planning was always carried out with two diametrically opposed fields provided with wedge filters in order to achieve uniform dose distributions in the affected region (Fig. 2b). When planning for several sections the tumour

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5 500—5 999	2/6	0/5	0/2	0/1		2/14	14.3
6 000—6 499	6/9	4/9	6/13	1/5	0/2	17/38	44.7
6 500—6 999	0/3	1/3	0/5	1/2	3/5	5/18	27.7
> 7 999	0/1		0/2	0/1	2/2	2/6	33
Total	8/21	7/20	6/22	4/11	5/9	30.83	36

were usually also used to determine the margins of the growth. These methods were combined with biopsy of the primary tumour. Thin needle aspiration biopsy (FRANZEN 1968) was always carried out when lymphatic gland metastases were possibly present in the neck. Direct extension of the growth into the regional lymphatic glands in the neck sometimes occurred when the primary tumour was advanced. It was then often impossible to determine whether or not the changes were due to lymphatic gland metastases. The doubtful cases were included among the lymphatic gland metastases.

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The percentage contribution from each field was obtained for each of the

Table 6

Survival after treatment of carcinoma of the hypopharynx

	With lymph node metastasis of the neck (57 patients)	Without lymph node metastasis of the neck (31 patients)
1 year survival	42/52 (40 %)	21/31 (68 %)
2 year survival	15/52 (29 %)	15/31 (48 %)
3 year survival	7/49 (14 %)	10/30 (33 %)
More than 5-year survival	6/50 (15 %)	5/25 (20 %)

Table 7

Survival after treatment of carcinoma of the hypopharynx with different techniques

	Decacurie (18 patients)	Kilocurie (65 patients)
1 year survival	10/18 (56 %)	32/65 (49 %)
2 year survival	6/18 (33 %)	24/65 (37 %)
3 year survival	3/18 (17 %)	14/61 (23 %)
More than 5-year survival	3/18 (17 %)	8/47 (17 %)

eighty three patients treated with ^{67}Ca 27 patients were subject to operation as follows

Tracheotomy (in a total of 16 patients)

Before treatment	4
During or less than 2 months after treatment	1
More than 2 months after treatment	11

Neck dissection (in a total of 9 patients)

Before treatment	1
During or less than 2 months after treatment	1
More than 2 months after treatment	7

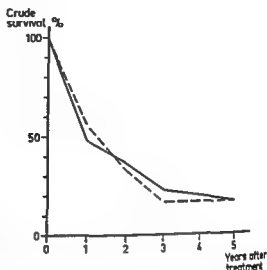
Gastrostomy (in a total of 11 patients)

Before treatment	2
During or less than 2 months after treatment	4
More than 2 months after treatment	5

Laryngectomy

In all cases more than 2 months after treatment	6
-------------------------------------------------	---

Fig 4 Survival curves for 18 patients treated with the decacurie technique (---) and 65 patients treated with the kilocurie technique (—). Two thirds of the patients in each group had cytologically verified lymph node metastases before receiving therapy and the 5 year survival rate for both groups was about 17 %



dose in each section was normalised to the tumour dose in the section through the centre of the tumour. The reference doses and the planned values were checked with small condenser ionisation chambers. The central doses for these sections could be determined by measuring the entrance and exit doses for various sections.

The treatment was given with a Siemens Gammatron 1 in the range 2 500—1 500 Ci (HULTBERG et coll 1959). The collimator consisted of a number of tungsten plates which could be introduced into the beam individually in order to obtain irregular beams and to shield particular areas; for instance, the larynx.

Before the first treatment was given, check films of the treatment area were exposed, using the cobalt apparatus. This checking is now performed with a roentgen simulator. Prior to each treatment and in the course of the treatment, particularly when changes have been made, the limits of the fields are controlled by fluoroscopy and a roentgen film with the simulator.

A tumour dose between 6 000 and 6 500 rad was usually given (Table 5) over 5 to 6 weeks to one field a day and with a daily tumour dose of between 200 and 250 rad. Mucosal reaction of the hypopharynx was generally observed 2 to 4 weeks after the commencement of treatment. At the end of treatment and during the weeks immediately following, a moderate epithelial reaction over the affected area was observed. The patients under treatment received comprehensive diet, generally fortified with vitamin injections and when sideropenia occurred, iron injections. A number of patients were also given antibiotic prophylaxis during a period immediately subsequent to treatment. It was usually possible to avoid acute oedema and thus tracheotomy or gastrostomy. In the

Table 8

Survival after treatment of carcinoma in different parts of the hypopharynx

	Upper hypopharyngeal carcinoma (63 patients)	Lower hypopharyngeal carcinoma (12 patients)	Carcinoma of both parts (8 patients)
1 year survival	32/63 (51 %)	7/12 (58 %)	4/8 (50 %)
2 year survival	27/63 (43 %)	4/12 (33 %)	4/8 (50 %)
3 year survival	12/59 (20 %)	3/12 (25 %)	2/8 (25 %)
More than 5 year survival	9/49 (18 %)	1/8 (13 %)	1/8 (13 %)

one of these patients survived for more than 6 years and three for more than 2 years after the radiation treatment. All the nine patients are now dead, the cause of death in all instances being malignancy.

The 5 year survival for the patients without lymph node metastases was thus 20 % and for those with confirmed lymph node metastases 15 %.

No definite differences in survival rates could be noted in this series between patients who received decacurie treatment and those who had kilocurie treatment as may be seen from Fig. 4 and Table 7.

The three patients surviving more than 5 years have died of intercurrent disease 8 to 9 years after the ^{60}Co treatment.

The results of the treatment of the patients with tumours situated in the upper and lower parts of the hypopharynx, respectively, are summarized in Fig. 5 and Table 8. For the patients with tumours in the upper part of the hypopharynx the 5 year survival rate was approximately 18 %; in the other groups it was smaller.

Discussion

The previously mentioned predisposing factors were sideropenia, the Plummer-Vinson (Patterson-Kelly) syndrome and misuse of alcohol and tobacco. Few of these factors previously so significant in Sweden were recorded in the present material. The Plummer-Vinson syndrome is a condition occurring predominantly in middle aged women, rarely in men. Anaemia is common unless sideropenia is present. Our material included only 19 women, most of whom were elderly. Six women and five men, however, had a moderate degree of sideropenia with serum iron values between 0.015 and 0.089 mg-% at the commencement of treatment. A more typical Plummer-Vinson condition with dysphagia, atrophic changes in the buccal, glossopharyngeal and oesophageal membranes, as well as spoon-shaped nails were noted in some of these patients.

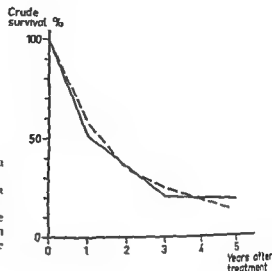


FIG. 1. Survival curves for 63 patients with carcinoma of the upper part of the hypopharynx (—) and for 12 patients with carcinoma of the lower part of the hypopharynx (---). The 5 year survival rate for the patients with tumours originating in the upper part was about 18 % and for those with tumours in the lower part of the hypopharynx was about 13 %.

Each patient was examined by a radiotherapist and an otolaryngologist before radiation treatment was commenced. All the patients had regular follow up examinations at Karolinska sjukhuset, with intervals between 2 and 6 months up to June 30th 1968. No patient was missed in the follow up and for 65 of the patients treated during 1963 or earlier the follow up was at least 5 years.

Results

The results for all the patients treated with ^{60}Co are summarized in Fig. 3. The 5 year survival in the whole series was about 17 %. Nine patients died of intercurrent disease, four of them within 5 years of treatment. The total 5 year survival rate after correction for the mortality due to intercurrent disease was approximately 31 %. Two of the eleven patients who were alive 5 years after the treatment were known to be suffering from recurrences or metastases and these were removed by operation, in one patient the operation took place at 1 and 3 years and in the other patient 6 and 8 years after the primary radiation therapy. Nine of the eleven survivors had no known recurrences or metastases when examined 5 years after treatment: one had suffered a relapse and another patient had a fresh primary tumour.

The survival times for the patients without and with verified lymph node metastases in the neck at the commencement of treatment are given in Fig. 3 and Table 6.

One of the six patients who survived more than 5 years had bilateral lymph node metastases before treatment. Nine patients had bilateral cervical metastases,

were 64 men and 19 women. It is particularly interesting that for the 52 patients with lymph node metastases confirmed by cytologic examination, the 5 year survival rate was 15 %. No equivalent material has yet been reported for hypopharyngeal tumours in the neck.

In earlier material from the same clinic, when roentgen irradiation was used there is no record during the period 1939—1942 of any patient with metastases in the neck who survived as long as 4 years. During the period 1943—1947 there were only four patients out of 165 treated with conventional roentgen radiation who survived 4 years or more. LEDERMAN (1962) and LEDERMAN & MOULD (1968) reported a 5 year survival rate of 7 % for patients with lymph node metastases. In our material the 5 year survival rate for patients with confirmed lymph node metastases was 15 % (see p 139). This was not much lower than for patients without confirmed metastases in the neck (20 %, see p 139 and Fig 3). It should be noted that only one of the patients who survived over 5 years in spite of cytologically confirmed lymph node metastases before treatment belonged to the group which was operated upon during the follow up period. Although the number of patients was small the improved result can to a large extent be attributed to the ^{60}Co treatment. However one must bear in mind the fact that as a result of better diagnosis smaller metastases can be shown and verified and that an increasing number of prognostically favourable cases therefore come into the group with confirmed metastases. In the present material lymph node metastases in the neck were not an excessively unfavourable sign when the method of treatment was the one described. One of the nine patients with bilateral cytologically confirmed metastases in the neck survived without relapse for 5 years after treatment.

The development of puncture cytology has also implied that in the frequent follow up studies that took place in this material it was possible to reveal recurrences at a much earlier stage than was possible previously. This has increased the possibility of using radical surgical methods to save these patients. For the 2 year survival the results were not dependent on the dose or fractionation times used (see Table 5). This has also been reported by other workers.

CLEMENT *et coll* (1964) see also the results reported by KURTIC (1963) and WOOD (1959). — It is difficult in our material to draw any conclusions from the comparison between the patients treated with the decacurie technique and those treated with the kilocurie technique. Those who were treated according to the former technique were specially selected although twelve of the eighteen patients had confirmed lymph node metastases. Of the sixty five patients treated with the kilocurie technique there were forty who had cytologically confirmed lymph node metastases prior to the commencement of radiation therapy. Thus two-thirds of those who had decacurie therapy as well as those who received

Dietetic factors are of importance in the development of the Plummer Vinson syndrome (JACOBSSON 1961, WANDER *et coll* 1957). Iron and vitamin C deficiency has been fairly common among women in certain parts of Sweden and this syndrome has often been encountered in the northern regions. Great improvements in the general diet has occurred in recent decades in Sweden however. Since 1938 all white flour has been fortified with iron and vitamin B complex. These prophylactic measures have probably been successful in reducing the incidence of the Plummer Vinson syndrome and thus carcinoma of the hypopharynx.

Carcinoma of the lower part of the hypopharynx is especially common with the Plummer Vinson syndrome. About 75 % of the hypopharyngeal carcinomas in the previously published series from this hospital, were situated at the lower part of the hypopharynx. Only 12 of the 75 carcinomas in the present series were in this location. In eight patients (Tables 3 and 4), however, the tumour was growing at both the upper and lower parts of the hypopharynx.

As regards tobacco and alcohol the records were less certain. There were, however, seven clear cases of ethylism all in men. The composition of the patient series was relatively unfavourable, with 25 tumours arising in the sinus piriformis, 18 large tumours that invaded adjacent organs, and 52 of the 83 patients had confirmed lymph node metastases prior to treatment. However, the total 5 year survival was approximately 17 %. A total of 84 % of the patients with carcinoma of the upper hypopharynx were men and 67 % of those with carcinoma of the lower hypopharynx were women. This corresponds well with the material previously reported from other clinics (JACOBSSON 1951). It should be pointed out first that carcinoma of the upper part of the hypopharynx occurred mostly in men, in whom the prognosis is always considered worse than in women, and secondly that the tumours were often extensive at the commencement of treatment. The latter was usually due to the fact that tumours arising in the piriform fossa often present few symptoms and therefore reach a more advanced degree of growth before diagnosis.

For a comparison with the results previously reported by JACOBSSON (1951) from Radiumhemmet it must be borne in mind that the composition of the material has changed over the years. During the years 1939 to 1947 the tumours in about three quarters of the patients were located in the lower part of the hypopharynx while in 63 of the 83 patients in the present series the tumours were in the upper part of the hypopharynx. This is undoubtedly connected with alterations in the etiology of the tumours described above and with the distribution between the sexes. In the period 1939—1947 there were 119 men and 203 women, while in the series treated with ^{60}Co during the period 1955—1965 the distribution between the sexes was reversed i.e. there

l'hypopharynx supérieur environ de 18 %. Le taux de survie pour 59 malades avec de mé tastases du cou vérifiées cytologiquement était de 15 %. Le pronostic était défavorable pour cette série qui comprenait 64 hommes et 19 femmes dont l'âge moyen était d'environ 60 ans

REFERENCES

- CLÉMENT R, AMALARIC R, SANTAMARIA F et DISTRIA J C Influence de la dose et de l'étalement sur la survie d'un groupe de cancers du larynx traités par télécobalt *J Radiol Électrol* 45 (1964) 859
- CUNNINGHAM M P and CATLIN D Cancer of the pharyngeal wall *Cancer* 20 (1967) 1959
- DALBY J E Treatment of post-cricoid carcinoma by combined intracavitary and external irradiation *Clin Radiol* 15 (1964) 251
- FRANZÉN S Studier över vardet av punktionscytologi vid diagnostik av tumorsjukdomar (in Swedish) Thesis Stockholm 1968
- HIDSON W R and CAVANAGH P J Combined surgical and radiation management of carcinoma of the laryngopharynx *Laryngoscope* 75 (1965) 1123
- HULTBERG S, DAHL O, THORAEUS R et coll Kilocurie cobalt 60 therapy at the Radiumhemmet Equipment technique and dose measurements *Acta radiol* (1959) Suppl No 179
- JACOBSSON F Carcinoma of the hypopharynx A clinical study of 322 cases treated at Radiumhemmet from 1939 to 1947 *Acta radiol* 35 (1951) 1
- The Paterson Kelly (Plummer Winson) syndrome and carcinoma of the cervical oesophagus *In* Tumours of the oesophagus Monographs on neoplastic disease Volume IV E & S Livingstone Edinburgh and London 1961
- KUTTIG H Telekobalttherapie der Geschwulste des Larynx und Hypopharynx. Erfahrungen und Behandlungsergebnisse *Strahlentherapie* 122 (1963) 493
- LEDERMAN M Carcinoma of laryngopharynx Results of radiotherapy *J Laryng* 76 (1962) 317
- and MOLLOY R F Radiation treatment of cancer of the pharynx with special reference to telecobalt therapy *Brit J Radiol* 41 (1968) 251
- MACCOMB W, HEALEY JR J E, MCGRAW J P et coll Hypopharynx and cervical esophagus *In* Cancer of the head and neck p 213 Edited by W E MacComb and G H Fletcher Williams & Wilkins Baltimore 1967
- RINGERTZ N, ERICSSON J, SJÖSTRÖM Å and SWENSSON D Cancer incidence in Sweden 19 9—1963 National Board of Health The Cancer Registry Stockholm 1962—1967
- SILVERSTONE S M, GOLDMAN J L and ROSEN H D Combined therapy irradiation and surgery for advanced cancer of the laryngopharynx *Amer J Roentgenol* 90 (1963) 1073
- WALSTAM R Studies on therapeutic short-distance and intracavitary gamma beam techniques Physical considerations with special reference to radiation protection *Acta radiol* (1965) Suppl No 236
- WOOD C A D Techniques and early results of treatment of carcinoma of the larynx and pharynx by supervoltage radiation *Brit J Radiol* 32 (1959) 661
- WYDER E L, HULTBERG S, JACOBSSON F and BRÖSS I J Environmental factors in cancer of the upper alimentary tract A Swedish study with special reference to Plummer Winson (Paterson Kelly) syndrome *Cancer* 10 (1957) 470

hilocuric treatment had verified lymph node metastases (see Fig. 4)

Preliminary reports on small selected materials have recently indicated a new way to improve the prognosis. For instance, SILVERSTONE *et coll.* (1963), DALBY (1964), HUDSON & CAVANAGH (1965) give as definite planned treatment a 5 500 rad tumour dose with ^{60}Co over a period of 5 weeks followed by radical surgery within 3 to 6 weeks. They have reported a 2 year absolute survival rate of 72 % and this must be considered a remarkably good result even for a small selected material. CUNNINGHAM & CATLIN (1967) maintain that radiation therapy alone cannot cure advanced pharyngeal carcinoma but that combinations of surgery and radiation therapy may save a few cases not controllable by any one of the methods alone.

Primary radical surgery, which has been prescribed by some workers has considerable disadvantages in the treatment of hypopharyngeal tumours. Even if the average age involved is comparatively high, and in spite of the fact that ^{60}Co therapy can be quite trying for the patient, no mortalities during the treatment have been recorded. With laryngectomy, with or without neck dissection, on the other hand, the operation mortality is comparatively high and the consequences for the patient are more severe.

SUMMARY

An analysis of a total of 83 patients with hypopharyngeal carcinoma treated with ^{60}Co during the period 1955—1965 is presented. The treatment methods are discussed. The total 5 year survival rate was approximately 17 % and in patients with upper hypopharyngeal carcinoma it was about 18 %. The survival rate was 15 % for 52 patients with cytologically verified metastases in the neck. The series was prognostically unfavourable with 64 men and 19 women and an average age of about 60 years.

ZUSAMMENFASSUNG

Eine Analyse von 83 Patienten mit Karzinom des Hypopharynx, die in den Jahren 1955—1965 mit ^{60}Co behandelt wurden, wird vorgelegt. Die Behandlungsmethoden werden besprochen. Die totale 5 Jahre Überlebenszeit war ungefähr 17 % und in Patienten mit Karzinom des oberen Teiles des Hypopharynx 18 %. Die Überlebensrate war 15 % in 52 Patienten mit zytologisch bestätigten Metastasen im Hals. Die Serie war prognostisch ungünstig mit 64 Männern und 19 Frauen und einem Durchschnittsalter von ungefähr 60 Jahren.

RÉSUMÉ

Un total de 83 malades atteints de cancer de l'hypopharynx a été traité par le ^{60}Co au cours de la période 1955—1965. Les auteurs discutent les méthodes de traitement. Le taux de survie total à cinq ans était approximativement de 17 % et pour le cancer de

Table 1

International comparisons or calibrations of secondary standards of other countries with the DAW cavity ionization chambers

Country	Place of measurement	Institution	Time	Type of measurement
Hungary	Budapest	Oncological Institute Budapest	July 1964	Measurement of ^{60}Co Unit Gravimeter
Bulgaria	Berlin	Oncological Institute Sofia	Jan 1965	Calibration of Siemens dosimeter unit
CSSR	Prague	Institute for Nuclear Research Prague	May 1963	Measurement of ^{60}Co source for the high pressure chamber to be constructed
CSSR	Berlin	Institute for Nuclear Research Prague	Nov 1966	Calibration of two chambers as secondary standards
Hungary	Berlin	State Board of Metrology Budapest	Nov 1966	Calibration of two chambers as secondary standards

deleeff Institute Leningrad U S S R (VNIIM) These comparisons are of special interest for two reasons Firstly they enable a comparison to be drawn between two methods of determining exposure i.e. the free air and cavity ionization chambers Secondly because a large number of countries have had their secondary standards calibrated by one or other of the centres reporting this inter-comparison For instance DAW cavity chambers have been used to calibrate commercial chambers of Czechoslovakian Hungarian and Bulgarian institutions as secondary standards because these countries still lack the facilities for absolute measurements (Table 1) Furthermore NPL standard chambers have been compared with primary standard chambers of the NBS Washington (Wickoff et coll 1963) NBS have calibrated a Swedish secondary standard and so on So these comparisons will give some idea of the accuracy of the roentgen unit as used in a larger number of countries

Ionization chambers for the absolute determination of exposure

The cavity ionization chambers of the Institute of Biophysics of DAW Berlin Buch The realization of the unit of exposure in the Institute of Biophysics of DAW is accomplished with a number of graphite cavity chambers with effective

INTERNATIONAL COMPARISONS OF IONIZATION CHAMBERS FOR ABSOLUTE MEASUREMENTS OF THE ROENTGEN AT PHOTON ENERGIES OF ABOUT 1 MeV

by

A RAKOW, W WIL, M YUDIN, G OSTROMUCHOVA, R KONOVA
A R S MARSH and J W G DALE

Accuracy of dose determination is of great importance for many applications of ionizing radiations in radiation therapy, radiobiology, and radiation biophysics. The absorbed dose (rad) is at the moment generally determined by measuring the ionization in air and multiplying this by a conversion factor. For example in the U K there is a code of practice for the dosimetry of high energy roentgen and gamma ray beams which gives conversion factors for particular types of chambers calibrated with 2 MV roentgen rays (Hospital Physicists Association 1964). Calibrations of commercial ionization chambers designated for use as secondary standards are carried out at regular intervals by the various state organizations using their own particular radiation standards.

The purpose of this paper is to report a direct comparison between the cavity ionization chambers of the Institute of Biophysics of the German Academy of Sciences of Berlin, G D R (DAW) with those of the National Physical Laboratory, Teddington U K (NPL) and with the pressure chamber of the Men

by means of plastic inserts so that a reproducibility of the control readings within ± 0.1 per cent is attained. The ionization measured per unit mass of dry air was corrected by a correction factor of the form

$$A = A_{Br} A_{SA} A_L A_{St} A_S$$

where

A_{Br} = correction factor for the different electron stopping power of the chamber wall material and air

A_{SA} = correction factor to take into account the attenuation of the primary radiation in the chamber wall

A_L = correction factor to take into account the non uniformity of the primary radiation intensity over the active chamber volume through wall attenuation and through geometric attenuation

A_{St} = correction factor to take into account scatter from the chamber stem

A_S = correction factor for extrapolation to complete saturation

These corrections so far have been determined for the experimental conditions existing at DAW Berlin-Buch (RAKOW & WILL 1963). For the conditions at NPL Teddington and VNIIM Leningrad some of the corrections could be determined directly by experiment and the shift of the remaining corrections could be estimated.

The comparisons were carried out with one of the DAW graphite cavity chambers which permits absolute measurements of the exposure and with a secondary standard chamber of air equivalent material (WILL & RAKOW 1970). Table 2 gives data of interest about these chambers. Table 3 contains corrections introduced into the measuring values of the graphite chamber under various conditions of irradiation.

Calibration chambers of the NPL Teddington. The primary standard in the UK for exposure measurements with 2 MV roentgen rays is based upon three cylindrical graphite cavity chambers of nominal volume 2 cm³ (BARNARD, AXTON, BELCHER & MARSH 1956). The chambers are mounted integrally on duralumin stems permanently connected to lengths of non-microphonic co-axial screened cable. The polarizing potential applied to these chambers is 300 V and their wall thickness is 500 mg/cm.

The chambers in turn are connected to one of a pair of Townsend balancing and measuring systems incorporating an electrometer triode valve bridge and standard capacitor. A whole beam parallel plate monitor chamber is permanently connected to the other system.

Table 2

Details of the two DAM-chambers used for the intercomparisons

Chamber	C 15 No 2	H 111/15 No 1
Measuring volume	1 507 cm ³	About 15 cm ³
Material	Graphite	Air equivalent material
Wall thickness	3.4 mm = 580 mg/cm ²	3.4 mm = 590 mg/cm ²
Chamber voltage	400 V	400 V
Sensitivity according to DAM calibration at $t=22^\circ\text{C}$, $p=760$ mm Hg, $RH=0\%$ complete saturation	} 4.60×10^{-16} C/R	4.91×10^{-16} C/R

Table 3

Correction factors for the DAM cavity chamber C 15 No 2 under different conditions of irradiation

		Corrections for the conditions of irradiation at		
		DAM	NPL	VNIM
k_{pr}	Stopping power	1.004	1.004	1.004
k_{sch}	Attenuation by total wall thickness	1.014	1.015	1.015
$k_{\text{U}}^{\text{Sch}}$	Irregularity of the intensity by attenuation	0.997	0.997	0.997
k_{U}^{S}	Irregularity of the intensity by geometry	0.995	0.999	0.995 (SCD=10 cm)
k_{st}	Stem scatter	0.997	0.997	0.997
k_{s}	Saturation	1.003	1.001	1.000
Total correction factor		1.011	1.013	1.008 ± 0.010

Maximum uncertainty of absolute exposure determination ± 2

volumes of from 1 cm³ to 2 cm³, the design of which has already been described (RAKOW & WILL 1963). The wall thickness of the chambers is varied by adding or removing close fitting graphite caps, for ⁶⁰Co γ radiation the thickness is 580 mg/cm². During measurement the chambers are connected via a co-axial cable to an electrometric photocell compensator (Townsend compensator), whose measuring capacitor, as well as the precision voltmeter and the thermometer and barometer for air density determination have been calibrated by the DAMW (DAMW = Deutsches Amt für Meßwesen und Warenprüfung). The chamber polarizing potential is 400 V. The constancy of chamber sensitivity is checked by a ⁹⁰Sr check source in which the constant location of the chambers is ensured

therefore a value 1.022 has been used in the comparisons now reported. This is close to the value 1.020 used in an international comparison in 1961 (ICRU 1964).

Estimates of the individual factors are given in Table 4. For the last correction factor f_1 , mutually compensating terms are thought to make its most likely value 1.000, but this factor has the greatest degree of uncertainty associated with it. The present estimate of the limits of uncertainty in the overall correction factor 1.022 is ± 0.008 . The NPL wish to point out that they are at present reviewing the values of these factors and intend to publish new estimates when the work has been completed.

High pressure standard ionization chamber of the Mendeleeff Institute for Metrology (Leningrad) The primary standard of the U.S.S.R. for the energy range from 0.3 MeV to 3 MeV is a parallel plate high pressure ionization chamber with aluminum strips as potential guides. The high voltage plate is 75 cm long, the collecting electrode 25 cm long and the two guard electrodes each 30 cm long. All electrodes are 40 cm wide and the plate separation is 40 cm. The beam entrance window consists of 5.77 mm thick duralumin. The design of the chamber was described by AGLINTSEV et coll. (1961). The air pressure within the chamber may rise to a maximum of 20 atmospheres and the chamber voltage to as high as 15 kV. The ionization current is measured by Townsend compensator.

Ionization measured per unit mass of air is corrected by the following factor

$$\Lambda = \Lambda_1 \Lambda_2 \Lambda_3 \Lambda_4 \Lambda_5 K_6$$

where

Λ_1 = correction for radiation attenuation in the inlet window of the tank

Λ_2 = correction for radiation attenuation by air layer between the inlet window of the tank and the centre of the measuring electrode

Λ_3 = correction for absence of saturation conditions in the standard chamber

Λ_4 = correction for penetration of radiation through the diaphragm edges

Λ_5 = correction for displacement of the effective region of gamma radiation absorption relative to the measuring volume

K_6 = correction for influence of the scattering radiation in air

The magnitudes of the individual factors are listed in Table 5.

Sources of radiation

The ^{60}Co teletherapy unit of DAB, Berlin-Buch The measurements in Berlin-Buch were carried out on the Theratron Junior ^{60}Co irradiation unit of the

Table 4

Correction factors for the NPL 2 cm³ standard graphite cavity chambers for 2 MV roentgen rays

$\frac{(m''en)_{\text{air}}}{(m''en)_{\text{carbon}}}$	0.999
$S_{m, \text{air}}^{\text{carbon}}$	1.003
f_{10}	1.018
f_e	1.002
f	1.000
Total correction factor	1.022 ± 0.008
Maximum uncertainty of absolute exposure determination $\pm 1.5\%$.	
For the NPL calibration service the value of f is taken as 1.008 in order to return a total correction factor of 1.03 as discussed on p. 148	

An exposure measurement in roentgens by the NPL chambers is given by

$$I \left[\frac{(m''en)_{\text{air}}}{(m''en)_{\text{carbon}}} S_{m, \text{air}}^{\text{carbon}} f_{10} f_e f \right]$$

I = charge in coulombs collected per 2.58×10^{-4} kg of dry air

$\frac{(m''en)_{\text{air}}}{(m''en)_{\text{carbon}}} =$ ratio of the mass energy absorption coefficients

$S_{m, \text{air}}^{\text{carbon}} =$ stopping power ratio carbon to air including polarization effect

$f_{10} =$ correction factor to take into account the attenuation due to the presence of the chamber wall

$f_e =$ correction factor to take into account lack of electronic equilibrium

$f =$ composite correction factor to take into account lack of saturation, configuration factors and stem scatter contribution etc

When the NPL initiated their 2 MV calibration service in 1956, the overall correction factor (the value of the expression in square brackets) was taken to be 1.03. However, by the year 1959 evidence had accumulated that this was probably an overestimate possibly by as much as 1 per cent (BARNARD & MARSH 1959, BARNARD, MARSH & HITCHMAN 1964). Nevertheless, the value 1.03 has so far been retained for routine calibrations in the interests of consistency, bearing in mind that it still lies within the limits of uncertainty associated with the value now thought to be more nearly correct. There is no reason however, why, in international comparisons a more recent estimate should not be taken, and

Table 6

Results of the comparison of the cavity ionization chambers of DAW and NPL

Chamber	C 15 No 2	H III/15 No 1
Sensitivity as determined by NPL (uncorrected)	(a)* 4.639**	4.934
C/R $\times 10$	(b) 4.640	4.935
	(c) 4.641	4.931
Mean	4.640	4.933
Sensitivity as determined by NPL (corrected)	4.54	4.83
C/R $\times 10$		
Sensitivity as determined by DAW (uncorrected)	4.65	4.97
C/R $\times 10$		
Sensitivity as determined by DAW (corrected by DAW for NPL conditions) C/R $\times 10$	4.59	4.90
Ratio of uncorrected sensitivities NPL/DAW	1.00	0.99
Ratio of corrected sensitivities NPL/DAW	0.99	0.99

The comparison was carried out on three separate occasions

**All sensitivity values quoted are for dry air at 22 °C and 760 mm Hg

Table 7

Results of the comparison of the cavity chambers of DAW with the high pressure ionization chamber of NIM

Chamber	C 15 No 2	H III/15 No 1
Sensitivity as determined by NIM C/R $\times 10$	(a) 4.54**	4.93
	(b) 4.49	4.87
	(c) 4.58	
	(d) 4.63	
Mean	4.56	4.90
Sensitivity as determined by DAW (corrected by DAW for NIM conditions) C/R $\times 10$	4.60	4.91
Ratio of sensitivities NIM/DAW	0.99	1.00

The comparison was carried out on four separate occasions

**All sensitivity values quoted are for dry air at 22 °C and 760 mm Hg

Table 5

*Correction factors introduced when measuring ^{60}Co exposure rate with the VNIIM standard device for 0.3 to 3 Mrad **

k_1	1.085
k_2	1.074
k_3	1.073
k_4	0.950
k_5	0.992
k_6	0.988
Total correction factor	1.164 ± 0.013

Maximum uncertainty of absolute exposure determination $\pm 1.5\%$.

*Conditions: chamber air pressure 10 atm, temperature 20°C, chamber field strength 440 V/cm.

Robert Roessle Clinic of the DAW. The measurements were made at the rotation centre of the unit (SCD 55 cm) to eliminate the distance error through irradiation with the two opposite positions of the source. The field size was 15 cm \times 15 cm at a distance of 55 cm, and the exposure rate under these conditions on 1 November 1966 was 55 R/min.

The 2 MV roentgen source of the NPL Teddington. The NPL uses a roentgen ray beam produced by a 2 MV Van de Graaff generator, having a tungsten transmission target and a focal spot of approximately 1 mm diameter. The diaphragm system was described by ASTON & SMITH (1954) and this produces a canalized beam, circular in cross-section. The broad spectrum of radiation can be represented by an equivalent spectrum with only three monoenergetic components (BARNARD et coll. 1959). More recently (BARNARD, MARSH & HITCHMAN 1964) good experimental agreement was reached with spectra calculated according to KRAMERS' method (1923).

For the measurements described in the paper, an aperture size was chosen so as to give a beam diameter of 4.5 cm at the calibration distance of 180 cm so that the ionization chambers were completely enveloped. The HVT of the roentgen beam was 12 mm Cu and the exposure rate at 180 cm was approximately 12 R/min.

^{60}Co radiation source of the primary standard of the VNIIM Leningrad. The ^{60}Co gamma emitter used for the comparison measurements in Leningrad with an activity of 4.5 Ci on 1 June 1966 (diameter 4 mm, height 4 mm, surrounded by a capsule of stainless steel of 1 mm wall thickness), is placed into a lead container of 35 cm diameter in a special holder. The diameter of the emerging beam

meters of 30 and 60 mm (corresponding to field diameters of 120 and 240 mm at a distance of 60 cm) The results of the comparison are presented in Table 7 The considerable scatter of the measured values is presumably due to the inaccuracy in determining the chamber source distance at the time of that comparison The results showed that the response of the DAW chambers obtained at the two institutes differed by not more than 1 per cent

Conclusion

The final results of the comparisons between the primary ionization chambers of the three countries involved are summarized in Table 8 Using correction factors thought by the respective centres to be most appropriate these results show agreement to about 1 per cent This is within the estimated limits of accuracy claimed by each of the laboratories It is reasonable to suppose therefore that the realization of the unit of exposure by means of high pressure and cavity ionization chambers is accurate to within about 2 per cent This provides confirmation of the validity of secondary standard calibrations for other countries carried out by the three centres concerned in the present intercomparisons

Acknowledgements

The comparison carried out at the NPL was based upon the standards and corrections established principally by Dr G P BARNARD to whom the authors would like to dedicate their contribution in memoriam

SUMMARY

The primary standard cavity ionization chambers of DAW Berlin were compared with the primary standard high pressure ionization chambers of VNIIM Leningrad using their ^{60}Co unit and with the primary standard cavity ionization chambers of NPL Teddington using their 2 MV Van de Graaff roentgen ray generator With correction factors thought by the respective centres to be most appropriate the comparisons showed agreement to about one per cent which is within the estimated limits of accuracy claimed

ZUSAMMENFASSUNG

Hohlraumionisationskammern der DAW Berlin wurden mit der Primärstandard Druckluft Ionisationskammer des VNIIM Leningrad an einer ^{60}Co Einheit und mit den Primärstandard Ionisationskammern des NPL Teddington an dessen 2 MV Van de Graaff Generator verglichen Unter Verwendung der von den entsprechenden Institutionen bestimmten Korrekturfaktoren zeigen die Vergleich Übereinstimmung auf etwa ein Prozent was innerhalb der Fehlergrenzen liegt

Table 8

Summary of international comparisons on DAW Chamber C 15 No 2

Standardising laboratory	NPL		VNIIM	
	NPL	DAW	VNIIM	DAW
Measurement or correction by				
Mean uncorrected sensitivities $C/R \times 10^6$	4.64	4.65	5.31	4.64
Mean corrected sensitivities $C/R \times 10^6$	4.54	4.59	4.56	4.60

can be varied by using lead diaphragms of from 30 mm to 60 mm diameter at a distance of 15 cm from the source. Radiation scattered in the container and diaphragm with 30 or 60 mm diameter of the diaphragm is 2 or 4 %, respectively (TUGIN et coll 1965). The exposure rate at 50 cm distance was about 0.3 R/min on 1 June 1966.

Results of the intercomparisons

Comparisons with the cavity ionization chambers of the DAW and the NPL
Comparative measurements with the cavity ionization chambers of the two institutions were carried out at NPL during September 1965 and March 1967. The same charge measuring apparatus including standard capacitor was used for all chambers and the intercomparison was carried out via a whole beam monitor chamber. All the chambers used in the experiments were unsealed, and, because it was assumed that all the chambers responded equally to changes in ambient air conditions, there was no need to apply any corrections for different ambient air conditions in the experiments. The cavity chambers of both institutions were of similar size so that no corrections for lack of beam uniformity were necessary.

From this intercomparison it appears that the ratio of the response of the DAW chamber C 15 No 2 to the mean response of the NPL chambers is unity. For the air equivalent chamber H III/15 No 1 this ratio is 1.01. If the corrections to the chambers as determined by their owners are applied then the exposure as determined by NPL is 1 per cent greater than that determined by the DAW chambers. These results are listed in Table 6.

The deviations in determining exposure are thus attributable to the respective differing corrections that are used.

Comparison between the cavity ionization chambers of DAW and the high pressure chamber of VNIIM
The comparison measurements were made in May 1966 at source chamber distances of 60 cm and 80 cm and for diaphragm dia

meters of 30 and 60 mm (corresponding to field diameters of 120 and 240 mm at a distance of 60 cm) The results of the comparison are presented in Table 7 The considerable scatter of the measured values is presumably due to the inaccuracy in determining the chamber source distance at the time of that comparison The results showed that the response of the DAW chambers obtained at the two institutes differed by not more than 1 per cent

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SUMMARY

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RÉSUMÉ

Les auteurs ont comparé les chambres d'ionisation à cavité étalons primaires de la DAW de Berlin avec les chambres d'ionisation étalons primaires à haute pression du VNIIM de Léninegrad au moyen de leur unité ^{60}Co et avec les chambres d'ionisation à cavité étalons primaires du NPL de Teddington au moyen de leur générateur de rayon de roentgen de Van de Graaff à 2 MV. En tenant compte des facteurs de correction considérés comme les meilleurs par les différents centres ces comparaisons ont montré une concordance à environ un pour cent près ce qui est dans les limites de précision attendue.

REFERENCES

- ACLINTZEW A. K. and OSTROMUCHOVA G. P. Wosproiswedenie rentgena w oblasti gamma isluscheniya s energiei kwantow ot 0.25 do 3 MeV. Trudii VNIIM 55 (1961), 115.
- ASTON G. H. and SMITH E. E. A comparison of γ ray standards in the United Kingdom. In Reports submitted to the International Commission on Radiological Units (ICRU), Copenhagen July 1953 p. 7. Acta radiol. (1954) Suppl. No. 117.
- BARNARD G. P. and MARSH A. R. S. A stopping power correction for graphite cavity chambers used with 2 MV γ rays. Phys. in Med. Biol. 4 (1959) 33.
- ASTON E. J. and MARSH A. R. S. A study of cavity ion chambers for use with 2 MV γ rays equilibrium wall thickness wall absorption correction. Phys. in Med. Biol. 3 (1959) 366.
- MARSH A. R. S. and HITCHMAN D. G. I. Studies of cavity ionization chambers with megavolt γ rays. Phys. in Med. Biol. 9 (1964) 295.
- ASTON E. J., BELLCHER D. S. G. and MARSH A. R. S. The present status of calibrations of ionization dosimeters with high voltage γ rays. Phys. in Med. Biol. 1 (1956) 18.
- HOSPITAL PHYSICISTS ASSOCIATION. A code for the dosimetry of 2 to 8 MV γ ray and $\text{Cs } 137$ and $\text{Co } 60$ γ ray beams. Phys. in Med. Biol. 9 (1964) 457.
- ICRU Report 10b (1962). PHYSICAL ASPECTS OF RADIATION. Handbook No. 85 National Bureau of Standards. Washington 1964.
- KRAMERS H. A. Theory of γ ray absorption and of the continuous γ ray spectrum. Phil. Mag. 46 (1923) 836.
- RAKOW A. and WILF W. Absolute Darstellung der Röntgeneinheit im Energiebereich der ^{60}Co γ Strahlung mit Hohlraumionisationskammern. Kernenergie 6 (1963) 496 und 546.
- TUGIN V. N., OSTROMUCHOVA G. P. and YUDIN M. F. O wlyani kollimirovuscheshchego ustroistwa pri graduiruwke i powerke rentgenmetrow dosimetrow s pomoschtschu obratnogo zowuch gamma islutschnikow. Izmeritelnaya tekhnika 3 (1965) 58.
- WILF W. and RAKOW A. Zur Messung der Standardionendosis in festen und flüssigen Medien bei Röntgen- und Gamma Strahlung mit Energien zwischen 50 keV und 3 MeV. V. Untersuchungen zum Einfluss der Luftäquivalenz von Ionisationskammermaterialien. To be published in Strahlentherapie.
- WYCKOFF H. O., ALLISY A., ASTON G. H. et coll. Intercomparison of national roentgen and gamma ray exposure dose standards. Acta radiol. Ther. phys. Biol. 1 (1963) 47.

PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF RADIOSTRONTIUM IN MICE

Dose effect relationship in ^{90}Sr induced bone tumours

by

AGNAR NILSSON

In spite of a very extensive literature concerning the carcinogenic effect of radiostrontium there seem to be only a few reports dealing with the various problems related to dose dependency. Studies in this field (FIVKEL *et coll* 1957) have mostly been concerned with the relationship between dose and frequency of mice having overt macroscopically detectable osteosarcomas. Many questions still remain which seem to be of interest for a better understanding of the dynamics of tumour formation and its relation to dose and time. In a previous report the main attention was focussed on the ^{90}Sr induced carcinomas and their localisation in the mucous membranes of the head, their development and relation to dose (NILSSON 1968). A more detailed study concerning the effect of blood and blood forming tissues and their dependency on dose will be presented in a further paper.

The purpose of the present work was to obtain more information concerning the dose-dependent histologic changes in bone and their relation to frequency, location and type of bone tumours. The relation between dose, time of appear

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Table 1
Experimental conditions and the ^{90}Sr doses employed

Dose of ^{90}Sr in $\mu\text{Ci/g}$ body weight	Total number of mice investigated	Number of mice killed in groups of five	Last day for sacrifice	Number of spontaneously dead mice
1.6	120*	65	300	50
0.8	121	75	360	46
0.4	122	95	480	27
0.2	122**	103***	540	17
Control	95	94****	570	1

Out of these animals five* and two** were lost during the experiment only three*** and four**** animals respectively were sacrificed in the last test group

ance and type of histologic changes and fluctuations in the bone cell populations was studied, as well as their possible relation to later development of tumours. The influence of the ^{90}Sr dose on tumour growth rate and latency time was also taken into account.

Material and Methods. Four groups of CBA mice, 75 days old, were treated intraperitoneally with $^{90}\text{Sr}(\text{NO}_3)_2$. A group of 95 animals not treated with ^{90}Sr were used as control. At intervals of 7, 14, 21 and 30 days after injection of ^{90}Sr , and then at monthly intervals, five mice from each were selected at random and sacrificed, until all mice in each series had been utilized. The experimental conditions and the ^{90}Sr doses employed are recorded in Table 1. The animals were examined roentgenologically before being killed and the roentgenograms were used as a guide for locating tumours.

The mice were sacrificed by cervical dislocation. Both femurs, tibiae and humerus, the spine, pelvic bones and the head were fixed in Steeve's fluid and decalcified in 20% formic acid for histologic examination. Conventional methods were used, the sections being stained according to the van Gieson method, haematoxylin-eosin, Lillie's azure-eosinate, Masson's trichrome method, PAS according to Hotchkiss and Foot and Foot's silver method. The animals that died spontaneously were autopsied and treated in the same way.

The numbers of osteoblasts and osteoclasts were determined per millimeter endosteum lining the epiphyseal-metaphyseal part of the distal femur, at various intervals of time between 7 and 210 days after injection of ^{90}Sr (Fig. 1). The bone sections were investigated in a Wild microscope equipped with a Wild Zeichentubus. The field was projected at a magnification of 1250 diameters and the endosteum was measured with an odometer. At each interval, 10 fields



Fig 1 Epiphyseal plate and metaphyseal bone of femur 30 days after injection of $0.4 \mu\text{Ci } ^{90}\text{Sr/g}$ body weight. Osteoblasts and osteoclasts were counted in a zone immediately below the epiphyseal cartilage (van Gieson $\times 175$).

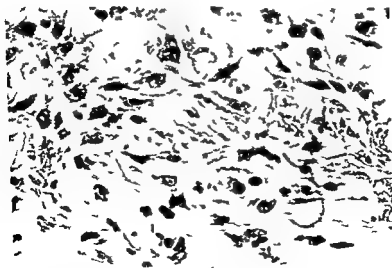


Fig 2 Non-neoplastic fibroblast proliferation in bone marrow femur 210 days after injection of $0.8 \mu\text{Ci } ^{90}\text{Sr/g}$ body weight (van Gieson $\times 50$).



Fig 3 Non neoplastic focal proliferation of osteoblast like cells and osteoclastic activity along the endosteal linings in diaphysis tibia 218 days after injection of $0.4 \mu\text{Ci } ^{90}\text{Sr/g}$ body weight van Gieson $\times 500$



Fig 4 Solitary neoplastic bud inside non neoplastic fibroblastic tissue from lumbar vertebra 300 days after injection of $0.8 \mu\text{Ci } ^{90}\text{Sr/g}$ body weight van

Table 2

Cause of spontaneous death in relation to doses of ^{90}Sr administered per gram body weight

	1.6 μCi		0.8 μCi		0.4 μCi		0.2 μCi	
	No of mice	Mean survival days	No of mice	Mean survival days	No of mice	Mean survival days	No of mice	Mean survival days
Acute aplastic anaemia	6	18.2						
Chronic anaemia	9	263	5	226			1	308
Osteosarcoma	19	259.9	30	319.5	8	408.0		
Squamous cell carcinoma	16	282.5	3	338.3				
Leukaemia			3	250	11	264.5	4	251.3
Adenocarcinoma							1	402
Angiosarcoma			1	314			1	425
Fibrosarcoma							1	347
Liver rupture (hepatoma)							1	422
Liver rupture (carcinoma of the liver)								
Not settled			4	350.0	8	293.8	7	383.4
Total	50	238.7	46	312.5	27	316.0	17	357.8

were counted in each of five femurs. The number of cells per millimeter of endosteum was determined by dividing the aggregate number of cells counted by the total length of the endosteum measured for the fifty fields. The cells supposed to be osteoblasts were characteristically arranged in a one cell thick layer carpeting the bone surfaces. The cells were either columnar in shape or they were thin flattened or later on fusiform elements. The osteoclasts were large multi- or uni-nucleated cells present on or near bone and in process of absorption.

The number of nucleated chondrocytes in the epiphyseal cartilage were determined at the same time intervals as the mean value of 10 unit areas of 6,400 μ^2 from both femurs of five mice. Chondrocytes consisted of single or multiple cells residing in lacunae in the intercellular substance of cartilage.

The mice were fed during the experiment on a standard diet ad libitum and kept in the same room under similar environmental conditions.

Histologic evaluation

The histologic events leading to overt paraosteal tumour growth in this material were defined as follows:

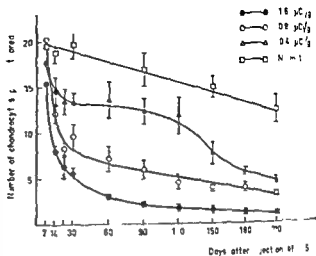


Fig 5 Number of chondrocytes per unit area ($6400 \mu^2$) related to dose and time after injection of ^{90}Sr . Confidence limit 67%

Non neoplastic proliferation Distinct proliferation of uniform reticular cells with a histologic appearance of fibrosis (Fig 2), with or without formation of foci of endosteal cell elements (Fig 3), in both crurs without neoplastic characteristics.

Osteosarcoma buds (microscopic tumours) Completely intramedullarily situated foci of proliferations containing pleomorphic cell elements (Fig 4). Buds of this type have earlier been shown to be transplantable and to have malignant characteristics (Nilsson 1962).

Overt macroscopic tumours Tumours breaking through the compact bone with or without infiltrating the surrounding soft tissue.

Results

A survey of the pathologic findings among spontaneously dead animals in the various dose groups are listed in Table 2. Some mice in the $1.6 \mu\text{Ci}$ group died early due to haematopoietic disorders. Of more interest is however that the animals succumbed from carcinomas nearly as often as from osteosarcomas. The cause of death in the $0.8 \mu\text{Ci}$ group was predominantly osteosarcomas, and in the 0.4 , and possibly also in the $0.2 \mu\text{Ci}$ group it was leukaemia. It should however be observed that many of the mice who died from carcinomas had osteosarcomas or leukaemia as well.

Pathologic features of the skeleton

Histologic appearances in relation to dose and time The variations in the distal femoral histologic appearances in relation to dose were distinguished by an

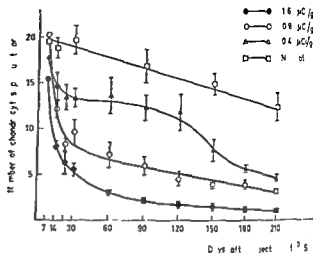


Fig 5 Number of chondrocytes per unit area ($6400 \mu^2$) related to dose and time after injection of ^{90}Sr Confidence limit 67%

Non neoplastic proliferation Distinct proliferation of uniform reticular cells with a histologic appearance of fibrosis (Fig 2), with or without formation of foci of endosteal cell elements (Fig 3), in both cases without neoplastic characteristics

Osteosarcoma buds (microscopic tumours) Completely intramedullarily situated foci of proliferations containing pleomorphic cell elements (Fig 4) Buds of this type have earlier been shown to be transplantable and to have malignant characteristics (NILSSON 1962)

Overt macroscopic tumours Tumours breaking through the compact bone with or without infiltrating the surrounding soft tissue

Results

A survey of the pathologic findings among spontaneously dead animals in the various dose groups are listed in Table 2. Some mice in the $1.6 \mu\text{Ci}$ group died early due to haematopoietic disorders. Of more interest is however that the animals succumbed from carcinomas nearly as often as from osteosarcomas. The cause of death in the $0.8 \mu\text{Ci}$ group was predominantly osteosarcomas, and in the 0.4 , and possibly also in the $0.2 \mu\text{Ci}$ group, it was leukaemia. It should however be observed that many of the mice who died from carcinomas had osteosarcomas or leukaemia as well.

Pathologic features of the skeleton

Histologic appearances in relation to dose and time The variations in the distal femur in histologic appearances in relation to dose were distinguished by an



Fig 8 Heavy destruction of epiphyseal plate and metaphyseal bone apposition of basophilic immature coarse fibred bone 270 days after injection of $1.6 \mu\text{Ci } ^{90}\text{Sr/g body weight}$ van Gieson $\times 300$

show the lowest incidence of these changes in the femur the $0.8 \mu\text{Ci}$ group the highest and the 0.4 group a frequency about four times as great as the 1.6 group

Intramedullary tumour buds The further development of the non neoplastic proliferations into intramedullary tumours consists of a successive confluence of expanding multicentric centres of proliferating osteoblast like cell elements. These cells attain in time a more atypical appearance lay down immature bone and acquire the histologic characteristics of an osteoblastic osteosarcoma. During neoplastic transformation of the fibroblastic type there seems to be constant formation of small solitary or multiple foci or accumulations of tightly packed atypical cells in the non neoplastic fibrous tissue (Fig 4). The induction time for intramedullary osteosarcomas was related to dose. Generally the osteoblastic type appeared earlier than the fibroblastic buds. On the other hand the mean induction time for the two tumour types did not differ significantly.

Rate of transformation For fibroblastic osteosarcomas there existed a dose related influence on the rate of transformation of non neoplastic proliferations into intramedullary osteosarcoma buds and further into overt paraosteal tumours. The rate of development was significantly increased in the $1.6 \mu\text{Ci}$ group as compared with the other series. No such relation was found for the osteoblastic type (Table 3).

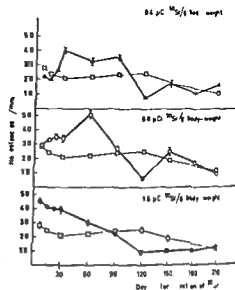


Fig 7 Number of osteoclasts per millimeter of endosteum in the distal metaphysis of femur related to dose and time in the controls

activity of the osteoblast population, endosteal foci of proliferating osteoblast like elements appeared. Such foci were seen in the 0.8 μCi group after 180–210 days but not until 300 and around 500 days, respectively, in the 0.4 and 0.2 μCi groups. These types of changes were not so striking in the 0.4 μCi group, instead, increased activity and proliferation of reticular cells predominated.

The histologic changes described were also studied in other bones. The destructive changes inside each group were generally of about the same magnitude in the tibia and femur but usually of much less severity in other bones, particularly in thoracic and cervical vertebrae.

Tumour development

Non neoplastic proliferations The occurrence of scattered, focally situated, proliferations of osteoblastic cell elements in resorption cavities or along endosteal linings (Fig 8) as well as a diffuse increase of reticular cells (Fig 2) could be detected in all the different dose groups. The relationship between dose and the first appearance and the rate of transformation of these changes into tumour entities was studied more thoroughly for the fibroblastic types of tumour since the different stages of their development are more clearly defined and easier to follow than the osteoblastic types (SUNDELIN & NILSSON 1968). The frequency and start of the changes were dependent on dose and time after injection of ^{90}Sr (Fig 9). In the 1.6 μCi group these changes were usually most numerous in the lumbar vertebrae and pelvic bones. In the other groups the long bones, particularly the femur, were most often involved. The 0.2 and 1.6 μCi groups

Table 3

Influence of dose on rate of tumour development calculated from curves in Fig. 8 by horizontal retrograde extrapolation from the points representing respectively detected osteosarcoma buds to the curves for detected non neoplastic proliferations and from macroscopic tumours to the curves for detected tumour buds

Dose	Tumour	Horizontal retrograde extrapolation of time interval between	
		Detected tumour buds and curve representing non neoplastic proliferations days \pm SE	Detected macroscopic tumours and curve representing tumour buds days \pm SE
1.6 μ Ci	Fibroblastic	21.3 \pm 4.3 (n. 19)	29.2 \pm 1.6 (n. 5)
0.8		33.1 \pm 0.9 (n. 66)	57.2 \pm 2.4 (n. 20)
0.4		66.1 \pm 3.9 (n. 50)	63.1 \pm 8.8 (n. 8)
1.6 μ Ci	Osteoblastic		53.3 \pm 2.7 (n. 30)
0.8			53.6 \pm 1.9 (n. 54)
0.4			63 \pm 17 (n. 6)
1.6 μ Ci versus 0.8 μ Ci	Fibroblastic	t = 8.364 (p < 0.0005)	9.722 (p < 0.0005)
0.8 μ Ci versus 0.4 μ Ci		t = 1.990 (p < 0.025)	0.648 (not significant)

Tumour bearing animals: tumour incidence and tumour multiplicity. The highest frequency of tumour bearing mice amongst both spontaneously dead (89.1%) and sacrificed animals (76.7%) was found in the 0.8 μ Ci group (see Table 4). More than half the number of mice in this group had macroscopic tumours while considerably fewer macroscopic tumours were seen in the other groups.

The total number of tumours in the various groups can be directly compared since the total number of mice was the same in each group (Table 4, Fig. 10). A total of 609 tumours were found in the whole material. Of this total number 35.9%, 47.9%, 14.8% and 1.3% were located in the groups which started with the highest dose. Of these tumours 37 (17.1%) in the 1.6 μ Ci group and 79 (27.1%), 17 (18.9%) and 1 (12.5%) in the lower dose series respectively were of macroscopic size. For all but the lowest dose group a considerable tendency towards occurrence of multiple tumours was observed (see Table 5).

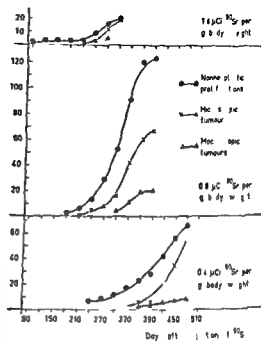


Fig 9 Accumulated number of fibroblastic non neoplastic proliferations microscopically intramedullary and macroscopically extramedullary fibroblastic osteosarcomas

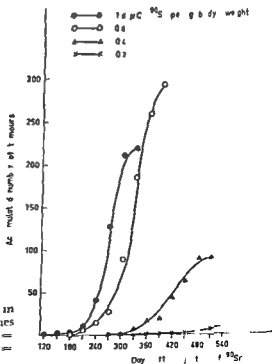


Fig 10 Total number of tumours occurring in the different dose groups. The latency times were: 1.6 μCi group = 267.6 ± 4.6 , 0.4 μCi = 320.7 ± 5.4 , 0.2 μCi = 426.7 ± 9.8 , 0.1 μCi = 485.1 ± 37.8

Table 4 (cont)

0.8 μ Ci			0.4 μ Ci			0.2 μ Ci		
Total number of mice	Number of mice with tumour	Total number of tumours	Total number of mice	Number of mice with tumour	Total number of tumours	Total number of mice	Number of mice with tumour	Total number of tumours
46	41 (82%)	195	97	11 (40%)	20	17	1 (5%)	1
45	0	0						
5	1	5						
5	4	8	55	0	0			
5	3	7	5	1	1	60	0	0
5	5	17	5	0	0	5	1	1
5	4	20	5	3	6	5	0	0
5	5	40	5	2	3	5	0	0
			5	1	2	5	0	0
			5	5	20	5	0	0
			5	4	12	5	0	0
			5	5	26	5	0	0
						5	2	4
						3	2	2
7	3 (30%)	97	95	21 (22%)	10	103	5 (4%)	8
167			525			104		

increasing dose for tumour development also in the thoracic spine and the head. In the particular long bones there was a location pattern which was obviously related to dose. Most tumours in the 1.6 μ Ci group were located in the proximal ends of the femur, tibia and humerus. The same applied in the 0.8 μ Ci series as regard tibia and humerus but in the femur the distal metaphysis was the predominating site. In the 0.4 μ Ci group on the other hand the diaphysis of these bones was most often involved (Table 7).

The location of tumours in relation to the type of osteosarcoma differed with respect to their sites in the different parts of the skeleton. In all the dose

Table 4

Tumour frequency and total number of tumours in relation to dose of ^{90}Sr in $\mu\text{Ci/g}$ body weight and time

Spontaneously dead mice	1.6 μCi		
	Total number of mice	Number of mice with tumour	Total number of tumours
	50	42 (84.0%)	137
Sacrificed mice			
Day 7-90	30	0	0
120	5	1	1
150	5	1	1
180	5	1	1
210	5	3	5
240	5	5	22
270	5	5	28
300	5	5	29
330			
360			
390			
420			
450			
480			
510			
540			
Total sacrificed mice	65	21 (32.3%)	87
Per cent of sacrificed mice with tumours after appearance of first tumour		60.0	

Induction time As may be seen from Fig. 10 the latency period was considerably longer with decreasing dose though it may be difficult from an experiment of this type to calculate the true mean induction with certainty.

Tumour location A definite correlation existed between dose and tumour location (Table 6). In the 0.8 μCi group and the 0.2 μCi group more than 50% of the tumours were located preferentially in the long bones, particularly the femur. In contrast most tumours in the 1.6 μCi group were sited in the lumbar, sacral spine and pelvic bones (56%). There was a preference with

Table 4 (cont.)

0.8 μ Ci			0.4 μ Ci			0.2 μ Ci		
Total number of mice	Number of mice with tumour	Total number of tumours	Total number of mice	Number of mice with tumour	Total number of tumours	Total number of mice	Number of mice with tumour	Total number of tumours
46	41 (89.1%)	195	27	11 (40.7%)	20	17	1 (5.9%)	1
45	0	0						
5		5						
5	4	8	55	0	0			
5	3	7	5	1	1	60	0	0
5	5	17	5	0	0	5	1	1
5	4	20	5	3	6	5	0	0
5	5	40	5	2	3	5	0	0
			5	1	2	5	0	0
			5	5	20	5	0	0
			5	4	12	5	0	0
			5	5	26	5	0	0
						5	2	4
						3	2	2
75	73 (97.3%)	97	95	21 (22.1%)	70	103	5 (4.9%)	8
16			59.5			104		

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The location of tumours in relation to the type of osteosarcoma differed with respect to their sites in the different parts of the skeleton. In all the dose

Table 5
Tumour multicentricity in relation to dose

Dose group $\mu\text{Ci/g}$ body weight	Number of osteosarcomas/mouse		
	Spontaneously dead	Sacrificed after appearance of first tumour	Whole experiment
1.6	2.6	2.5	2.6
0.8	4.2	3.2	3.8
0.4	0.74	1.8	1.3
0.2	0.06	11.6	0.13

Table 6
Anatomical distribution of osteosarcomas in relation to dose delivered in $\mu\text{Ci/g}$ body weight

Location	Per cent tumours in each dose group				Total number of tumours in each dose group			
	1.6 μCi	0.8 μCi	1.1 μCi	0.2 μCi	1.6 μCi	0.8 μCi	0.4 μCi	0.2 μCi
Femur	8.2	28.8	28.9	(25)	18	84	26	?
Tibia	5.9	13.4	14.4	(37.5)	13	39	13	3
Humerus	5.9	12.7	11.1	(12.5)	13	37	10	1
Total long bones	20.1	54.8	54.4	(75.0)	44	160	49	6
Pelvic bones	22.8	13.7	17.8		50	40	16	
Cervical spine	3.7	3.1	2.2		8	9	2	
Thoracic spine	6.8	2.1			15	6		
Lumbar spine	22.8	11.0	5.6		50	32	5	2
Sacral spine	10.5	5.5	11.1	(25.0)	23	16	10	
Lumbar and sacral spine	1.4	0.3			3	1		
Coccygeal spine	2.7				6			
Total vertebral column	47.9	21.9	18.9	(25.0)	105	64	17	2
Head	7.8	4.5	2.2		17	13	11	
Multiple site	1.4	5.1	6.7		3	15	6	
Total					219	292	90	8

Table 7

Distribution of tumours in various parts of the long bones in relation to the total number of tumours in each of the long bones within the dose group

μCi	Sr/g body weight	Total number of tumours	Distribution of tumours in					
			Distal epiphysis		Diaphysis		Proximal epiphysis	
			Number	Per cent	Number	Per cent	Number	Per cent
<i>Femur</i>								
1.6		18	1	5.6	4	22.2	11	61.1
0.8		84**	38	45.2	22	26.2	17	20.9
0.4		26	4	15.4	15	57.7	4	15.4
<i>Tibia</i>								
1.6		13	2	15.4	7	53.8	4	30.8
0.8		39	1	2.6	5	12.8	32	82.1
0.4		13*	0	0.0	8	61.5	3	23.1
<i>Humerus</i>								
1.6		13	3	23.1	1	7.7	9	69.2
0.8		37*	4	10.8	6	16.2	25	67.6
0.4		10	0	0.0	9	100.0	0	0.0

The number of tumours that could not be located to a particular zone are indicated as follows: one tumour () two tumours (*) three tumours () seven tumours ()

groups the humerus and the lumbar and the sacral spine had the highest frequency of fibroblastic osteosarcoma. In the pelvic bones there was a strong predominance of osteoblastic osteosarcoma in the 1.6 μCi group whereas in the 0.4 μCi group it was of fibroblastic type. All tumours in the head were of the osteoblastic type. As regards the site inside particular bones the fibroblastic intramedullary osteosarcoma buds in the 0.4 μCi group occurred to a much greater extent in the diaphyseal part of the long bones than did the osteoblastic tumour buds.

Tumour types With decreasing dose there was a shift from a predominance of osteoblastic to fibroblastic osteosarcomas (Table 8).

Discussion

Relationship between dose and tumour frequency in various tissues Each of the different ^{90}Sr doses administered in this investigation proved capable of inducing osteosarcomas. In previous studies (FINKEL 1958) the same was reported with

Table 8
Histological classification of bone tumours

Type of tumours	Percentage distribution by dose			
	1.6 μCi n 219	0.8 μCi n 292	0.4 μCi n 90	0.2 μCi n 8
Osteoblastic osteosarcomas	87.2	68.8	24.4	(50.0)
Fibroblastic osteosarcomas	11.0	29.8	71.1	(37.5)
Chondroblastic osteosarcomas	0.9	—	—	—
Angiosarcomas	0.9	2.1	4.4	(12.5)

doses even lower than we used. The optimal dose, 0.8 $\mu\text{Ci/g}$ body weight, was in good agreement with earlier reports (FINKEFL 1958, NILSSON 1962). The frequency of osteosarcomas was still very high in the 1.6 μCi series and, in addition, carcinomas occurred in the mucous membranes of the mouth and nose, as reported in a separate paper (NILSSON 1968). In the highest dose group, leukaemia was not detected among the spontaneously dead mice. This was the case in each of the other series, however, the highest frequency being in the 0.4 μCi group. The existence of a clear optimum of carcinomas, osteosarcomas and leukaemia for the doses 1.6, 0.8 and 0.4 $\mu\text{Ci/g}$, respectively, seems to indicate a strong dependency on dose and on the topographic location of the tissue involved. The radiation delivered from a certain dose of radiostrontium to the mucous membranes close to bone is much less than to the osseous tissues and to the bone marrow. Thus, doses inducing carcinoma may generally be too destructive for induction of osteosarcomas at a maximum rate, and even more so for induction of leukaemia. Only doses with an effect not more destructive than allowing recovery and a certain degree of regeneration will promote an optimal frequency of neoplasia. It was also shown in an earlier investigation (NILSSON 1967) that female mice injected with an optimal osteosarcoma dose had a significantly reduced number of osteosarcomas when an increased excretion (30%) of ^{90}Sr was brought about by lactation. At the same time, however, there was a significant increase of leukaemia compared with non lactating mice.

Relation between dose and latency time As previously demonstrated in this investigation, there was a correlation between dose and latency time not only for the first occurrence of neoplastic changes but also for the appearance of the destructive and regenerative phases and non neoplastic proliferations (Figs 9 and 10). This clearly indicates that the latency time is a function of dose and time.

In this relation the total accumulated dose seems to be a more decisive factor in tumour genesis than the initial dose rate (NILSSON et coll 1967, VAN PUTTEN 1961)

Rate of tumour development After becoming established as a stage of development the fibroblastic non neoplastic proliferations were more rapidly transformed into intramedullary fibroblastic osteosarcomas and to overt neoplasms within the higher dose groups (Table 4) There was thus a significantly more rapid development of the fibroblastic tumours in the 1.0 than in the 0.8 and 0.4 μCi groups The reason for this might be a combination of factors, such as the occurrence of more selectively altered, radioresistant cell clones after higher doses the growth of which may not be suppressed since the immunologic capacity and resistance of the host are diminished

The growth rate of osteoblastic buds was not enhanced by dose (Table 4) These tumour buds from the histologic point of view are more highly differentiated than the fibroblastic ones and consequently might not be influenced in the same degree by irradiation The reason for the general reduction of the mean induction time with increasing dose seems to be primarily the earlier occurrence of tumour cell clones and not a generally increasing growth rate

Relation between dose and tumour multiplicity It is seen from Table 5 that the multicentric genesis of tumours is a function of time and dose since with increasing survival there is a perpetual rise in the number of tumours in each group of animals

Considering the relation between tumours of macroscopic and microscopic extent it is of interest to note that the latter predominated strongly in the 0.8 μCi group Many microscopic tumours develop with a suboptimal not too destructive dose but the survival time for the mice is too short for their transition into macroscopic entities The induction time with a suboptimal dose might be too long for the development of an appreciable number of macroscopic tumours

Dose related histologic variations in bone tissue When comparing homologous bones between different dose groups, the degree and extent of histologic changes have been found to be strongly dose-dependent Such differences are however also obvious when comparing heterologous bones inside each group This variation seems to be related to the fact that the radiation dose absorbed is determined by many factors such as the size geometry and metabolic rate of the bones This may explain the more serious effect on the femur than on the lumbar vertebrae and why the latter are more damaged than the thoracic vertebrae

In order to transform the various radiation doses into terms of their mutual biological effect the nucleated chondrocytes in the epiphyseal plate of the distal

femur were counted (Fig 5). These cells were expected to provide a better reference than the osteoblasts to the dose relations in this part of the bone. Only in the 0.4 μCi group were there signs of recovery and a temporary regeneration. In the two other groups, and particularly in the 1.6 μCi group, there was a continuously enhanced cellular depletion with increasing dose. The osteoblastic and osteoclastic populations in the distal femur showed more variations (Figs 6 and 7). There was thus a continuously decreasing number of cells in the 1.6 μCi group. In the group given 0.8 μCi , which is the optimal osteosarcoma dose, the osteoblastic population was forced into a state of instability characterized by a diphasic fluctuation. The recovery observed after 60 days might be characterized as an abortive regeneration, which may indicate its origine from a cell population so heavily damaged that it could only survive a limited number of divisions. The second regeneration which started after about 180 days appeared to give birth to scattered, focal, non neoplastic proliferations (Figs 2 and 3) which, however, successively were transformed into tumours. This might indicate that cells originating from this second regeneration were selected, possibly quite radioresistant, but defective, and potentially malignant cell elements. The destructive effect of the highest dose will not allow any appreciable degree of regeneration in the distal femur, as mirrored by the low incidence of tumours in the latter. The 0.4 μCi dose, on the other hand, may not cause damage sufficient to induce the instability necessary in the osteoblastic population to create optimal conditions for tumour development. Of particular interest is however that the changes most prominent in the 0.4 μCi group were located in the reticular cells of the bone marrow to a much higher degree than in the other groups. These cells give rise predominantly to the fibroblastic type of osteosarcomas (Fig 4). Some observations made in the 0.4 μCi group as well have shown advanced destruction and necrosis in the bone marrow, including the reticular cells. This might be an expression of circulatory disturbances inside the bone marrow. A lesser radioresistance of the reticular cells compared with that of the osteoblastic elements might also explain why the osteoblastic elements more often proliferate in the higher dose groups.

Relation between dose and tumour location The importance of dose for the tumour location is illustrated in Table 7. There is a remarkable shift from a significant predominance of tumours in the long bones, particularly the femur, in the 0.8 and the 0.4 μCi groups to a clear underrepresentation in the 1.6 μCi series. The number of tumours in the latter group is thus only 28% of that in the 0.8 group. In the pelvic bones and the spine there are instead 20% and 39% more tumours in the 1.6 μCi than in the 0.8 μCi group. It should also be pointed out that with increasing dose there are more tumours located in such

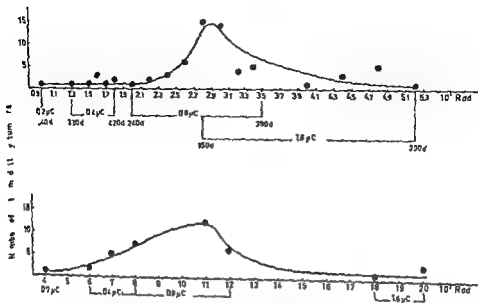


Fig. 11 Occurrence of intramedullary osteosarcomas in relation to the total accumulated dose in the different dose groups. Upper curve dose to the distal and proximal epiphyseal parts of the femur. Lower curve dose to the midshaft of the femur.

small bones as the thoracic and lumbosacral vertebrae. The same applies to the location of tumours in the head. This may be connected with the previously mentioned differences in the radiation doses absorbed in various parts of the skeleton. A comparison of the 0.4 and 0.8 μCi groups reveals a fairly constant pattern of tumour location.

The tumour sites inside particular bones also reveal a changed pattern with decreasing dose. About 25% of the tumours in the long bones of the 1.6 and 0.8 μCi groups were located in the diaphysis. In the 0.4 μCi series 73% were located there.

Relation between total accumulated dose in various bones and tumour frequency. It seemed of interest to investigate the influence of the total accumulated dose upon the tumour rate in the various dose groups. The femur was chosen as a model for this purpose. The doses absorbed in the distal epiphysis, the diaphysis and the proximal epiphysis of the femur were calculated. The mice 70 days old when injected and weighing 21 to 22 g were given 0.8 μCi ^{90}Sr g body weight and the weight of the femur and its various parts were determined. According to the investigation of PARMEY et al. (1962) the absorbed energy

in the whole femur was 38 %. The mean energy of ^{90}Sr — ^{90}Y is 1.13 MeV per disintegration. On the basis of these data and the retention curves for the femur, an approximate radiation dose to the various parts of the femur can be calculated. The doses in Fig. 11 are given merely as reference values and refer only to microscopic intramedullarily situated osteosarcomas. The doses in Fig. 11 (upper curve) relate to osteosarcoma buds located only in the proximal and distal parts of the femur, and those in Fig. 11 (lower curve) only to buds located in the central part of the diaphysis. There is a range between 9 500 and 52 000 rad, particularly between 27 000 and 31 000 rad, in which most tumours occur. This optimum can be assigned almost solely to the 0.8 μCi group, the dose in the 1.6 μCi group being too high for an optimal tumour induction in the femur.

In the diaphysis of the femur most tumours appeared around 10 000 to 12 000 rad. A possible explanation of this might be that the magnitude of the dose that induces irreversible changes leading to tumour formation is in fact much smaller than indicated by these figures and the figures for the distal and proximal parts. Tumours have exceptionally been observed after only 4 500 rad, and around 9 000 rad in the distal part. On the assumption that a minimum time, and thus a minimum accumulated dose, is necessary for induction of histologic changes successively leading to formation of tumours it seems probable that the dose delivered after this time does not have much importance. The dose is constantly increasing during the time it takes for a tumour to develop. This surplus dose will be higher at certain sites like the distal metaphysis of the femur, since the dose rate there is much higher than in the diaphysis. In some cases this surplus seems to have some effect in promoting the rate of development of fibroblastic osteosarcoma, at least in the highest dose group. No such effect was observed for the osteoblastic type, however. On the other hand, it must be borne in mind that a suproptimal dose might have a depressing effect initially on tumour induction and later also on its further development.

It may be of interest to note that the frequency of diaphyseal tumours in relation to the total number in the femur was 36 %. The dose delivered was 35 % of that in the distal and proximal epiphysis. As mentioned earlier, most tumours in the 0.8 μCi group occurred in the femur and in the 1.6 μCi group in the spine, particularly the lumbar vertebrae. It would have been of interest to compare the doses delivered to these bones but no such measurements have however been made. A rough approximation from retention curves of femur and lumbar vertebrae, and also from calculations made by MARSHALL & FINKEL (1959), reveals an estimated dose to the lumbar vertebrae of about 50 % of that delivered to the femur. This seems to be the explanation of the optimal tumour frequency in the spine for the highest dose level.

Tumour types The percentage of fibroblastic tumours was enhanced with decreasing dose. This observation is however contradictory to that of STRELTSOVA (1959) who reported an increasing number of osteoblastic (osteosclerotic) tumours with decreasing dose in rats. In the 0.4 μCi series this type of tumour had a tendency to be overrepresented in the diaphysis of the long bones (69%), where the radiation dose was only about 30% of that delivered to the metaphyseal parts of the bone. The mean tumour induction time on the other hand was about 160 days longer than in the 1.6 μCi group.

Some cases of angiosarcoma have been found in all the dose groups but only one chondroblastic osteosarcoma was detected in the 1.6 μCi group. The percentage distribution of angiosarcomas indicates a tendency to increase with decreasing dose.

SUMMARY

The dose dependency was investigated in four groups of male CBA mice injected intraperitoneally with respectively 1.6, 0.8, 0.4 and 0.2 μCi of $^{90}\text{Sr/g}$ bodyweight. A group of 15 animals was used as control. Five mice from each group were sacrificed at certain intervals. It was shown pathologically and by conventional histologic method that not only latency time and tumour frequency but also type, multicentricity, location and to some extent the growth rate of osteosarcomas were related to the dose. The pathologic changes preceding tumour development and their relation to the dose given are discussed.

ZUSAMMENFASSUNG

Die Dosisabhängigkeit wurde an vier Gruppen von männlichen CBA-Mäusen, die intraperitoneal mit bzw. 1.6 μCi , 0.8 μCi , 0.4 μCi und 0.2 μCi von $^{90}\text{Sr/g}$ Körpergewicht injiziert wurden, studiert. Eine Gruppe von 95 Tieren wurde als Kontrolle benutzt. Fünf Tiere aus jeder Gruppe wurden bei verschiedenen Intervallen getötet. Pathologisch und histologisch wurde gezeigt, dass nicht nur die Latenzzeit und Frequenz der Tumoren sondern auch Art, Multizentrität, Lage und in gewissen Masse Zuwachsraten von Osteosarcomen in der Dosis abhängig sind. Pathologische Veränderungen bevor Beginn der Tumorentwicklung im Verhältnis zur gegebenen Dosis werden diskutiert.

RÉSUMÉ

L'auteur a étudié l'influence de la dose sur quatre groupes de souris mâles CBA auxquelles ont été injectées par voie intrapéritonéale respectivement 1.6 μCi , 0.8 μCi , 0.4 μCi et 0.2 μCi de $^{90}\text{Sr/g}$ de poids corporel. un groupe de 95 animaux a servi de témoin. Cinq souris de chaque groupe ont été sacrifiées à certains intervalles. Les méthodes anatomo-pathologiques et histologiques habituelles ont montré que non seulement le temps de latence et la fréquence d'apparition des tumeurs mais aussi leur type, leur caractère multicentrique, leur siège et dans une certaine mesure la vitesse de croissance des ostéosarcomes sont liés à la dose. Les modifications anatomo-pathologiques qui précèdent le début du développement de la tumeur et leur relation avec la dose administrée sont étudiées.

REFERENCES

- FINKEL M P, BISKIS H and SCRIBNER G M The influence of strontium 90 upon life span and neoplasms of mice *In* Progress in nuclear energy Series VI Biological science p 199 Pergamon Press, New York 1959
- FELLESEN H J, LESTINA J and BISKIS H The influence of dosage pattern upon the toxicity of ^{90}Sr in mice Argonne Nat Lab (1957) Report ANL 5732 p 21
- MARSHALL J H and FINKEL M P Autoradiographic dosimetry of mouse bones containing Ca^{45} , Sr^{90} and Ra^{226} Argonne Nat Lab Radiological Physics Division (1959) Semi annual Report ANL 6104, p 48
- NILSSON A (a) Sr^{90} induced osteosarcomas *Acta vet scand* 3 (1962) 127
- (b) Histogenesis of ^{90}Sr induced osteosarcomas *Acta vet scand* 3 (1962), 185
- Influence of gestation and lactation on radiostrontium induced malignancies in mice I Incidence distribution and characteristics of ^{90}Sr induced malignancies *Acta radiol Ther Phys Biol* 6 (1967) 33
- Pathologic effects of different doses of ^{90}Sr in mice Development of carcinoma in the mucous membranes of the head *Acta radiol Ther Phys Biol* 7 (1968) 27
- NELSON A, RONNBOCK C et coll Influence of gestation and lactation on radiostrontium induced malignancies in mice II Retention of radiostrontium and relation between tumour incidence and excretion rate *Acta radiol Ther Phys Biol* 3 (1967) 129
- PARNLEY W W, JENSEN J B and MAYS C W Skeletal selfabsorption of beta particle energy *In* Some aspects of internal irradiation p 437 Pergamon Press New York 1962
- STRILTSOVA V N Principles governing the development of osteogenic sarcoma induced by radioactive isotopes *Probl Oncol* 5 (1959) 1
- SUNDELIN I and NILSSON A Cytoplasmic ultraviolet extinction of strontium 90 induced fibroblastic osteosarcomas correlated to histologic appearance and ultrastructure *Acta radiol Ther Phys Biol* 7 (1968) 161
- VAN PUTTEN L M Treatment of radiostrontium intoxication in mice II Survival and bone tumour frequency *Int J Radiat Biol* 5 (1961) 477

RADIOSENSITIVITY OF MEDIASTINAL LYMPHOMAS IN HODGKIN'S DISEASE TREATED WITH SPLIT COURSE RADIOTHERAPY

A retrospective study

by

TORSTEN LANDFBERG and HANS FORSLO

Radiotherapy has been the method of choice in the treatment of clinically local Hodgkin's disease since 1902 when PUSEY reported good results in malignant lymphomas. Several chemotherapeutics are now available but appear to be reserved mainly for patients in whom radiotherapy cannot be expected to produce the desired effect especially if the disease is advanced. The belief that Hodgkin's disease is in the end always fatal (PATERSON & PATERSON 1954) now seems to be giving way to the feeling that it is at least sometimes unicentric in origin and possibly curable by radiation treatment (KAPLAN 1952, 1966; EASSEN & RUSSEL 1963; JELLIFFE 1965 and EASSEN 1966) justifying irradiation of diseased tissues to adequate dose levels.

Opinions differ concerning the dose that should be given in the treatment of local Hodgkin's disease (Table 1). PITCOCK *et coll.* (1959) and FAYON *et coll.* (1963) have reported longer survivals among patients treated with larger doses.

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and it has been demonstrated (JELLIFFE & THOMSON 1955, NOETZLI & SHELINE 1962, JELLIFFE 1965 and WESTLINE 1965) that the number of recurrences after irradiation diminishes with increasing dose. KAPLAN (1966) presented a curve for different rates of recurrences at various dose intervals, based upon data from the literature which indicated that a dose of 4 000 rad over 4 weeks should be considered the tumoricidal dose in Hodgkin's disease. Time versus dose diagrams for irradiated patients with Hodgkin's disease without and with local recurrences have been presented by SCOTT (1961), SCOTT & BRIZEL (1964), JELLIFFE (1965), FRIEDMAN *et coll* (1967) and SEIDEL *et coll* (1967). SCOTT (1961) gave a time dose line that passed through 750 R in 0.45 days and 2 300 R in 20 days, the doses expressed representing the minimal tumor dose as estimated by the prescribing physician. SCOTT & BRIZEL (1964) compared orthovoltage and supervoltage techniques. The doses had been calculated as minimal tumor dose in tissue roentgens along the central axis of the beam. The patients received five treatments a week. The authors stated that successful treatment with the supervoltage technique required a significantly larger dose than did the orthovoltage technique. They also reported that the dose required for short treatment times is higher than might be expected. According to JELLIFFE (1965) considerable variations in the radiosensitivity of Hodgkin's disease exist, a view shared by FRIEDMAN *et coll* (1967) who also thought that in one and the same patient the radiosensitivity of one lymph node group might vary from another one. Megavoltage radiotherapy required a larger dose than the orthovoltage technique. They recommended 3 500 rad over 4 weeks and gave the dose as 'a tumor dose' but failed to add a precise definition of the term. SEIDEL *et coll* (1967) recommended a dose of 3 500 to 4 000 rad also over 4 weeks.

Special attention has been paid in certain reports to the irradiation of mediastinal lymphomas in Hodgkin's disease. HONIL *et coll* (1951) pointed out that successful irradiation of the mediastinum often needs a longer period of treatment and thus a larger dose. They recommended target doses of between 3 000 and 4 000 rad. NICE & STENSTROM (1955) found that mediastinal involvement in Hodgkin's disease did not worsen the prognosis more than did involvement of any peripheral lymph node group. These authors recommended a minimum of 2 000 tissue roentgens to the tumour over 14 days. LEVITT (1959) recommended 2 500 R over 14 days. NOETZLI & SHELINE (1962) studied 11 recurrences in 22 patients treated for mediastinal Hodgkin's disease. In nine of these, the tissue doses had been 2 000 R or less and the treatment period, 3 months or less. VAPTH (1962) investigated 45 cases of recurrent mediastinal Hodgkin's disease and suggested a target dose of 3 500 R but gave no treatment period. FULLER *et coll* (1967) recommended 3 500 to 4 000 rad over 4 weeks.

Table 1
Dose/time data recommended for treatment in Hodgkin's disease

Author (s)	Dose/time data
HOLL et al (1951)	3 000-4 000 rad/7
NICE & STENSTRÖM (1954-1955)	2 000 tissue roentgens/3 weeks
HEALY et al (1955)	1 500-2 500-4 000 R/1 to 3 weeks
JELLIFFE & THOMSON (1955)	3 500 R/3.5 to 4 weeks
ELKIN (1955)	3 000-3 500 R/2 weeks
PETERS & WIDDELOW (1958)	2 500-3 000 R/2 weeks
LEVITT (1959)	2 500 R/2 weeks
VAETH (1962)	3 500 R/7
ARTHACHINTA & OGDEN (1962)	2 000-2 800 roentgens/2 to 3 weeks
CROSBIE (1962)	3 500-4 000 R/3 to 4 weeks
NAPLAV (1962-1966)	3 500-4 000 rad/3 to 4 weeks
ELSON & RUSSEL (1963)	2 500-2 750 rad/3 weeks
SALEMAN et al (1964)	2 400-3 500 R/3 weeks
ANDERBERG (1964)	3 500 R/3.5 to 4 weeks
JELLIFFE (1965)	2 500-4 000 rad/2 to 4 weeks
NEWALL (1965)	2 000-2 500 rad/2 weeks
PASSON (1966)	3 000-3 250 rad/3 weeks
FULLER (1967)	4 000 rad/4 weeks
FULLER et al (1967)	3 500-4 000 rad/4 weeks
FRIEDMAN et al (1967)	3 500 rad/4 weeks
SAVDEL et al (1967)	3 500-4 000 rad/4 weeks

The total treatment time (STRANDQVIST 1944) as well as the total number of fractions (ELLIS 1963) in fractionated radiotherapy are important. Most studies on split-course radiotherapy have been made on human cancers or animal tumours. SCARLON (1963) however in a casuistic report of split course radiotherapy for mediastinal Hodgkin's disease reported good regression of the lymphomas in the interval between the two series after 1 350 R. HOLSTI (1966) found that the end results of split course radiotherapy of human cancers were not inferior to those after continuous irradiation though doses administered in the former cases were larger. HOLSTI had often noted shrinkage of the tumours during the interval between the two series. SAMBROOK (1963) claimed that treatment must be resumed before recovery of the mitotic activity in the tumour but added that this interval is unknown although he had never seen clinical evidence of renewed growth in human epitheliomas within the first 6 to 8 weeks after 2 000 to 2 500 R given over 2 to 3 weeks. DU SALT (1954) in a study using different fractionation types of irradiation of spontaneous mammary carcinoma in mice reported that continuous treatment produced better results than split

Table 2

Comparison of patients without and patients with clinically diagnosed recurrences in relation to sex, clinical stage, histologic type and fractionation — Median values and ranges are given for age and fractionation data

	Group A = Mediastinal recurrence not diagnosed clinically	Group B = Mediastinal recurrence diagnosed clinically
Males females	6 5	5 3
Age (years) at first mediastinal treatment	31 (15—48)	24 (16—45)
Clinical stage at first mediastinal treatment	$\left\{ \begin{array}{l} \text{I} \\ \text{II} \\ \text{III} \end{array} \right.$	$\left\{ \begin{array}{l} 0 \\ 7 \\ 1 \end{array} \right.$
Histologic type in biopsy specimen		
Lymphocytic predominance	3	1
Nodular sclerosis	4	5
Mixed cellularity	4	2
Lymphocytic depletion	0	0
Treatment period (days)		
First series	22 (11—32)	25 (17—37)
Second series	13 (6—17)	12 (8—54)
Totally	71 (56—89)	76 (61—131)
Number of fractions		
First series	20 (12—24)	21 (16—24)
Second series	12 (6—16)	8 (8—16)
Totally	32 (19—36)	32 (24—33)
Interval (days) between the series	38 (31—49)	41 (31—47)

course therapy. The later the interval in split course treatment the better were the results. Though no direct correlation could be shown, the end results tended not to be as good when the interval was as long as when it was short. SCANLON (1960) stated that the recovery time for tumours varies from patient to patient. FRIEDMAN *et coll.* (1967) produced an isoeffect recovery curve indicating that Hodgkin's disease was capable of recovering from the effects of radiation. This recovery resembled that of carcinoma more closely than that of a radiosensitive lymphoma, such as *mycosis fungoides*.

The aim of the present retrospective investigation was to find out whether any difference in the target dose level was demonstrable between patients without and patients with clinically diagnosed recurrences after initial radiation treatment for mediastinal Hodgkin's disease. It was decided to study the lymph node group in the mediastinum (1) because this region is often involved early in

progressive disease (LANDBERG & LARSSON 1968 1969), (2) because the geometrics for mediastinal irradiation is fairly well defined and (3) because the results of therapy may be verified by roentgen examination

Material and Follow up A total of 246 patients with Hodgkin's disease were referred to the department during the period 1944-1960, and 149 of these who were previously untreated, were admitted. Re examination of the biopsy specimens obtained before the beginning of treatment had invariably verified the diagnosis. The patients had been followed up by the system described by LINDHOLM (1962). The period covered by the present investigation allowed a follow up of at least 5 years from the commencement of treatment.

The material was divided according to the clinical staging of the disease (JELLIFFE & THOMSON 1955 JELLIFFE 1965)

Stage I Lymph node involvement in only one main group but not including intra abdominal disease

Stage II Lymph node involvement of 2 or more groups in the upper or lower half of the body but not including intra abdominal disease

Stage III Generalized lymph node involvement — intra abdominal involvement — involvement of structures other than lymphatics — constitutional symptoms for which no other reasonable cause can be found

The material was divided according to the histologic type of lesion (LUKES et coll 1966) as follows: lymphocytic predominance — nodular sclerosis — mixed cellularity — lymphocytic depletion. Further details of the materials are given elsewhere (LANDBERG & LARSSON 1969 LANDBERG 1969)

Fifty-six patients in all received radiation for mediastinal lymphomas (re irradiation for recurrence excluded). Lymphadenopathia at the site of treatment after the initial course of radiotherapy was regarded as a recurrence (KAPLAN 1966). Of the 56 patients 29 were excluded from this series: one in whom the treatment had been given in one series only, eighteen mostly with advanced disease who had been followed up for at the most one year after the treatment of the mediastinum and ten whose roentgenograms were not available for review. In none of the remaining 27 patients had any mediastinal recurrence been diagnosed clinically within less than 26 months of the first irradiation of the mediastinum. It was therefore decided to exclude a further eight patients who had been followed up for less than 26 months after the first mediastinal irradiation.

The series thus consisted of 19 patients with mediastinal Hodgkin's disease followed up for 3 years or more after the first treatment of the mediastinum. The examinations had revealed mediastinal recurrence in eight (group B) but not in the remaining eleven (group A) patients.

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Comparison of patients without and patients with clinically diagnosed recurrences in relation to sex, age, clinical stage, histologic type and fractionation — Median values and ranges are given for age and fractionation data

	Group A = Mediastinal recurrence not diagnosed clinically	Group B = Mediastinal recurrence diagnosed clinically
Males females	11 5	5 3
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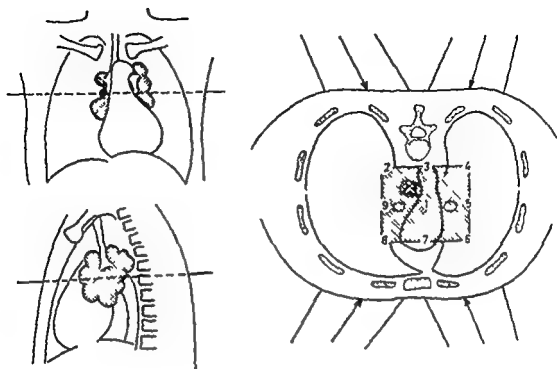


Fig 1 Example of reconstructed contour and of irradiation geometry in mediastinal Hodgkin's disease

Radiation data and estimation of the doses given: The treatment in all the nineteen patients had been given as split course therapy in two series (Table 2). Irradiation had been given in fifteen of the patients with 170 kV roentgen rays (HVL 0.9 mm Cu) and two ventral and two dorsal oblique portals, in one (No 158) with 170 kV roentgen and two ventral portals, in one (No 114) with 200 kV roentgen and rotation and in two (Nos 94 and 128) with ^{60}Co and one ventral and two dorsal portals. One field was irradiated daily and the treatment was given 6 days a week.

Information about doses in clinical materials should be founded on individual dose planning made at the time of treatment. Such precise information is, however, seldom included in clinical reports, and the various ways employed in reporting doses given to patients make it difficult to compare the therapeutic results obtained in different clinics (ELLIS 1963).

In the present material chest roentgenograms taken at the beginning of treatment and later during follow up were available. Reconstructions were drawn for all the nineteen patients of a contour through the centre of the mediastinal lymphoma by means of the roentgenograms that had been obtained at the beginning of treatment (Fig. 1). The roentgenographic enlargement factor was taken as 1.10 for AP views and 1.15 for lateral views. The error in such reconstructions

may be considerable in the lateral parts of the contour but can be kept within reasonable limits in the medial parts. In ten patients the reconstructed contours were checked either with contours made at the time of treatment or with those obtained at a review. The reconstructed contours in the medial parts in these ten patients agreed well; the difference in r.p. distance was found to be within ± 1 cm. It is apparent from Fig. 1 that only the medial parts of the contours are of interest in the further discussion.

The patient records contained information on the surface absorbed dose type and energy of radiation, geometric field sizes and angle of incidence. The dose delivered to nine different points (Fig. 1) was calculated with the assumption that the volume treated was comparable to water-equivalent tissue from the point of view of radiation absorption (Clinical Dosimetry, ICRU Report 10d, 1963). The dose to the target volume varied noticeably in the 4 portal technique (Fig. 1). In addition the lateral parts of the target volume were prone to fall outside the radiation fields even with slight variation of the angle of incidence and thus a minor variation in the set up could result in considerable changes in the distribution of the dose delivered. The patients were however young, mostly in good general condition, and presumably cooperated well. Roentgenologically the regression of mediastinal lymphomas is centripetal and this was noted in sixteen of the nineteen patients by the time the second series was started. When it occurs, the degree of reproducibility of the geometry probably becomes less critical.

The treatments have thus been expressed as the doses to the centres of the target volumes and disregard the dose variation in the peripheral parts of the target volumes.

Dose measurements were made in phantoms in order to estimate the error involved by the assumption that the irradiated tissue was water equivalent. A volume in a Machlett Alderson Rando Phantom (Machlett Laboratories Inc. Springdale, Connecticut, U.S.A.) with skeleton and simulated lung tissue was irradiated with the 4 portal technique (Fig. 1). A phantom of the same dimensions but consisting only of water equivalent material was then made and a corresponding volume was irradiated. Dose measurements were made with individually calibrated thermoluminescence dosimeters (lithium borate in teflon). The measured dose delivered to the centre of the target volume in the Alderson phantom was 111 ± 2 rad S.D. (overall uncertainty) based on the maximum absorbed surface dose of 100 rad for a single field. The corresponding figure for the homogeneous water equivalent phantom was 106 ± 2 rad S.D. The calculated dose to the centre of the target volume was 100 rad. If it be accepted that this experiment can be used as a guide in the evaluation of the doses calculated for the patients, then a correction of the calculated dose to the centre

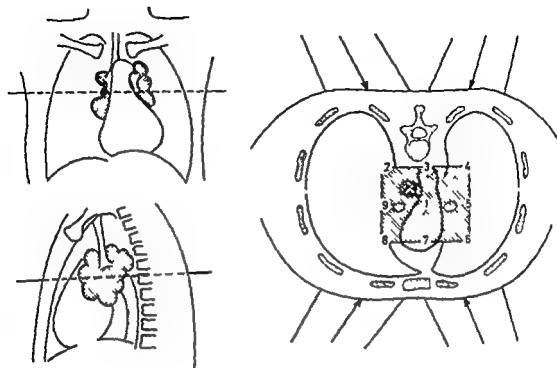


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Table 3

Treatment data for each one of the patients and interval between the first mediastinal irradiation and clinical detection of recurrence in patients with and without mediastinal recurrences — L denotes living patients and D those who died

	Patient No	Dose to centre of target volume (rad)	Total treatment period (days)	Total number of fractions	Follow up after first mediastinal irradiation (months)	Mediastinal recurrence after first irradiation (months)
Group A — Mediastinal recurrence not diagnosed clinically	29	4100	89	37	159 L	
	47	3600	82	36	122 D	
	58	4400	89	36	175 I	
	72	3900	87	36	150 L	
	74	5500	68	30	43 D	
	85	3700	71	37	137 L	
	94	5000	67	30	54 L	
	112	4200	61	28	104 L	
	114	3600	60	19	50 L	
	128	4400	74	33	60 L	
Group B — Mediastinal recurrence diagnosed clinically	158	3400	56	90	60 I	
	6	3500	83	33	197 D	92
	11	3900	131	32	39 D	26
	38	3400	77	37	83 D	51
	40	3400	75	30	56 D	51
	50	3100	80	32	81 D	75
	95	3000	61	28	95 D	73
	139	3500	75	32	93 L	73
	150	4300	66	24	58 D	35

of the mediastinal irradiation. The mediastinal recurrence was the first new sign of Hodgkin's disease after the irradiation of the mediastinum in two of the patients in group B (Nos 6 and 139).

Comparison between patients without and patients with recurrence in relation to dosage. A review of the roentgenograms obtained before completion of the irradiation of the mediastinum revealed changes in the pulmonary parenchyma or pleurae in five of the nineteen patients. The changes were located outside the irradiation fields in three patients (Nos 74, 95 and 150). Such changes though slight were seen within the irradiation field in two patients (Nos 112 and 114).

The data for each one of the patients are assembled in Table 3 giving the calculated dose to the centre of the target volume, the treatment period, the

of the target volume by reason of heterogeneous tissue should be in the order of $\pm 10\%$. We have, however, only indicated the calculated dose in the text. The correction factor for determination of the absolute dose should then be 1.1. A similar figure has been given by JACOBSON & KRAUFER (1955), who made measurements with ionization chambers in a phantom man made of presdwood and having a cork lung.

Results and Discussion

Comparison between findings in patients without recurrence and patients with recurrences irrespective of dosage. The second treatment series was prolonged to 54 days in one patient (No. 11-B), but otherwise the patients in group A and in group B were comparable (Table 2) as regards distribution according to sex, age, clinical stage, histologic type and fractionation. The height of the irradiation fields was 10 to 18 cm in the patients in group A and 11 to 22 cm in the patients in group B.

Three of the patients in group A (Nos. 47, 74 and 114) and one of the patients in group B (No. 11) had also extra mediastinal manifestations which had not been treated when the mediastinal irradiation was completed.

Roentgen examination before the beginning of the second series revealed no abnormality in four of the patients in group A (Nos. 29, 72, 74 and 94) and in one of the patients in group B (No. 150).

The mediastinal recurrences were diagnosed 26 to 92 months (mean 60) after the irradiation of the mediastinum. Of the eleven patients in group A, eight had been followed up for 5 years or more and six for more than 8 years after the beginning of the mediastinal irradiation. In the series of KAPLAN (1966) and of SFRIDEL *et al.* (1967) recurrences and extensions tended to occur within 2 to 3 years of the beginning of treatment of Hodgkin's disease. In the present series, at first all patients with a follow up of at the most a year were excluded; among the remainder recurrences were not diagnosed until 26 months or more after irradiation of the mediastinum had been started.

Treatment with cytotoxic drugs had been initiated before the first irradiation of the mediastinum in three of the patients in group A (Nos. 85, 114 and 158) but in none of the patients in group B. Later, five more patients in group A and all patients in group B received cytotoxics. Such treatment had been started in five of the eight patients in group B before the mediastinal recurrence had been diagnosed.

Further manifestations of Hodgkin's disease at other sites were diagnosed later in six of the patients in group A at 4 to 68 months (mean 32) and in all the eight patients in group B at 1 to 191 months (mean 42) after the beginning

ment period (Fig 2) and to the number of fractions (Fig 3). It is apparent that for a given number of days or with a given number of fractions recurrences were more common among patients who had received smaller doses. For a given dose level the frequency of recurrences seemed to diminish with a decreasing number of days and a decreasing number of fractions. It was not possible, however, to analyze the material in order to obtain a fractionation curve.

The presence or absence of a recurrence in the present material thus tended to vary with the calculated dose to the centre of the target volume in relation to the treatment period and to the number of fractions but not with any other of the factors studied. The treatments were administered within (median) 75 days and in (median) 32 fractions and it seemed that in these intervals the dose to the centre of the target volume should not be below 3 600 rad if it is to be effective. The dose would be about 4 000 rad with the correction factor of 1.1 for inhomogeneous tissue.

Autopsy was performed in one of the group A patients who had died (No 47) the cause of death was Hodgkin's disease. The examination revealed signs of the disease in a node in the mediastinum.

Acknowledgements

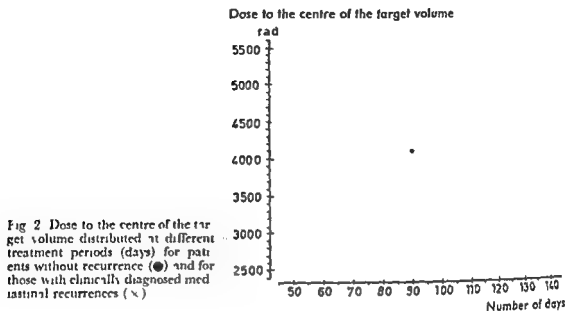
The authors wish to thank J. Bang of the Department of Roentgen Diagnosis for help with the re-evaluation of the roentgenograms. The work was supported by grants from the Swedish Cancer Society.

SUMMARY

Doses delivered to the centres of the target volumes were calculated retrospectively in nineteen patients followed up for at least 3 years after split course irradiation for mediastinal Hodgkin's disease. There were eleven patients without recurrences who were compared with eight patients in whom recurrences had been diagnosed clinically. The development or absence of a recurrence seems to depend mainly on the size of the dose to the centre of the target volume.

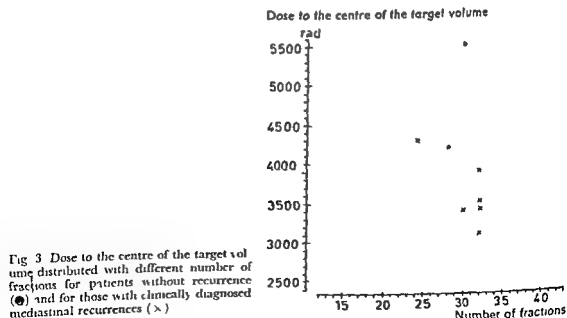
ZUSAMMENFASSUNG

Die am Zentrum des Bestrahlungsgebietes gelieferten Dosen in neunzehn Patienten mit Hodgkinscher Krankheit des Mediastinums, die für ein Minimum von 3 Jahren nach fraktionierter Tiefenbestrahlung unter Kontrolle gehalten werden konnten, wurden retrospektiv berechnet. Ein Vergleich konnte zwischen elf Patienten, die rezidivfrei waren, und acht Patienten, die klinisch Rezidive hatten, angestellt werden. Das Auftreten von Rezidiven scheint im wesentlichen von der Grösse der Dosis im Zentrum des Bestrahlungsgebietes abhängig zu sein.



number of fractions and the follow up and, for the patients in group B, also the recurrence free interval

The dose to the centre of the target volume for patients without and with clinically diagnosed recurrences is recorded in Figs 2 and 3, related to the treat



- Evidence for a tumoricidal dose level in the radiotherapy of Hodgkin's disease *Cancer Res* 26 (1966) 1221
- LANDBERG T Clinical course of Hodgkin's disease treated with radiotherapy *Acta radiol Ther Phys Biol* II (1969) 487
- und LARSSON L E Studium des klinischen Verlaufs bei Sternbergscher Erkrankung *Radiol Austr* III (1968) 197
- -- Hodgkin's disease Retrospective clinicopathologic study in 149 patients *Acta radiol Ther Phys Biol* 8 (1969) 390
- LEVITT W M Cancer of the reticulo-endothelial system *In Cancer Volume 5* p 363 Edited by R W Raven Buttersworth & Co London 1959
- LINDGREN M Organisation der Nachbehandlung und Kontrolle bestrahlter Geschwulst kranke *Radiologie* 2 (1967) 309
- LUKES R J BUTLER J J and HICKS E B Natural history of Hodgkin's disease as related to its pathologic picture *Cancer* 19 (1966) 317
- NEWALL J The management of Hodgkin's disease *Clin Radiol* 1965 1 p 40
- NICE C M and STENSTROM K W Irradiation therapy in Hodgkin's disease *Radiology* 67 (1954) 641
- -- Irradiation therapy in Hodgkin's disease of the thorax *Dis Chest* 28 (1955) 529
- NOETZLI M and SHELING G E Local recurrence in lymph nodes irradiated for Hodgkin's disease *In Progress in radiation therapy Volume 2* p 188 Edited by F Buschke Grune & Stratton New York and London 1962
- PATERSON R and PATERSON E Hodgkin's disease *Brit J Med* 2 (1954) 1315
- PETERS M V and MIDDLEMISS K C H A study of Hodgkin's disease treated by irradiation *Amer J Roentgenol* 79 (1958) 114
- PITCOCK J A BAKER W C and MCGAVRAN M H Hodgkin's disease in children *Cancer* 12 (1959) 1043
- PLATT W A Cases of sarcoma and of Hodgkin's disease treated by exposures to X rays A preliminary report *J Amer med Ass* 38 (1907) 166
- SALZMAN F A SNEED M I WRIGHT K A and TRUMP J G Two MeV wide field irradiation of lymphoma *Amer J Roentgenol* 92 (1964) 124
- SAMBROOK D K Clinical aspects of fractionation and dose time relationships *Brit J Radiol* 36 (1963) 1/4
- SCANLON P W Initial experience with split-dose periodic radiation therapy *Amer J Roentgenol* 84 (1960) 637
- Split-dose radiotherapy Follow up in 50 cases *Amer J Roentgenol* 90 (1963) 280
- SCOTT R M Time-dose relationship in Hodgkin's disease *Radiology* 76 (1961) 276
- and BAIZEL H E Time-dose relationship in Hodgkin's disease *Radiology* 82 (1964) 1043
- SEYDEL H G BLOEDORN F G and WIZENBERG M J Time-dose volume relationship in Hodgkin's disease *Rad ology* 89 (1967) 919
- STRANDQVIST M Studien über die kumulative Wirkung der Röntgenstrahlen bei Fraktionierung *Acta radiol* (1944) Suppl No 55
- VAETH J M Retreatment of mediastinal Hodgkin's disease *In Progress in radiation therapy Volume II* p 164 Edited by F Buschke Grune & Stratton New York and London 1962
- WESTLING P Studies of the prognosis in Hodgkin's disease *Acta radiol* (1965) Suppl No 245

RÉSUMÉ

Les auteurs ont calculé rétrospectivement les doses délivrées au centre des volumes cibles chez 19 malades suivis pendant trois ans ou moins après irradiation fractionnée pour maladie de Hodgkin médiastinale. Ils ont comparé onze malades sans récurrence et huit malades chez lesquels on avait fait le diagnostic clinique de récurrence. L'apparition ou l'absence d'une récurrence paraît dépendre surtout de la dose calculée au centre du volume cible.

REFERENCES

- ALLENBERG A C Hodgkin's disease. Prognosis, treatment and etiologic and immunologic considerations. *New Engl J Med* 270 (1964) 508
- ARTIAGUANTA S and ORDIN R T Hodgkin's disease. *Hartford Hosp Bull* 17 (1967) 103
- CLINICAL DOSIMETRY ICRU Report 101 National Bureau of Standards Handbook 87, Washington 1963
- CROSBIE J A clinical study of Hodgkin's disease. In *Progress in radiation therapy* Volume 2 p 153 Edited by I Buschke, Crane & Stratton New York and London 1962
- DUSAULT L A The influence of time spacing of fractions on response to radiation. *Amer J Roentgenol* 91 (1964) 90
- EASON F C Possibilities for the cure of Hodgkin's disease. *Cancer* 19 (1966) 345
- and RUSSEL M H The cure of Hodgkin's disease. *Brit med J* 1963 I, p 1704
- ELKINS H B Treatment of malignant lymphoma and blood dyscrasias by conventional roentgen therapy. *Amer J Roentgenol* 76 (1956) 960
- ELLIS F The dose time relationship in radiotherapy. *Brit J Radiol* 36 (1963) 153
- FAYOS J, HENDRIK R, MACDONALD V and LAMPE I Hodgkin's disease. *Amer J Roentgenol* 93 (1965) 557
- IRIDMAN M, PEARLMAN A W and IURELOV L Hodgkin's disease. Tumor lethal dose and iso effect recovery curve. *Amer J Roentgenol* 99 (1967) 843
- FULLER L M Results of intensive regional radiation therapy in the treatment of Hodgkin's disease and the malignant lymphomas of the head and neck. *Amer J Roentgenol* 99 (1967) 340
- , JING B S, SHULLENBERGER C C and BUTLER J J Radiotherapeutic management of localized Hodgkin's disease involving the mediastinum. *Brit J Radiol* 40 (1967) 913
- HEALY R J, AMORY H I and FRIEDMAN M Hodgkin's disease. *Radiology* 64 (1955) 51
- HOHL K, SARASIN P and BESSLER W Therapie und Prognose der Lymphogranulomatose. *Oncologia* 4 (1951) 1
- HOLST L R Split course megavoltage radiotherapy. One year follow up. *Brit J Radiol* 39 (1966) 332
- JACOBSON L F and KNAUER I S Correction factors for tumor dose in the chest cavity due to diminished absorption and scatter in lung tissue. *Radiology* 67 (1956) 863
- JELLIFIE A M The present place of radiotherapy in the cure of Hodgkin's disease. *Clin Radiol* 16 (1965) 274
- and THOMSON A D The prognosis in Hodgkin's disease. *Brit J Cancer* 9 (1955) 21
- KAPLAN H S The radical radiotherapy of regionally localized Hodgkin's disease. *Radiology* 78 (1962), 553
- Role of intensive radiotherapy in the management of Hodgkin's disease. *Cancer* 19 (1966) 356

- Evidence for a tumoricidal dose level in the radiotherapy of Hodgkin's disease *Cancer Res* 26 (1966) 1221
- LANDBERG T Clinical course of Hodgkin's disease treated with radiotherapy *Acta radiol Ther Phys Biol* 11 (1969) 487
- und LARSSON L E Studium des klinischen Verlaufs bei Sternbergscher Erkrankung *Radiol Austr* 18 (1968) 197
- — Hodgkin's disease Retrospective clinicopathologic study in 149 patients *Acta radiol Ther Phys Biol* 8 (1969) 390
- LEVITT W M Cancer of the reticulo-endothelial system *In Cancer* Volume 5 p 363 Edited by R W Raven Butterworth & Co London 1959
- LINDBERG M Organisation der Nachbehandlung und Kontrolle bestrahlter Geschwulst kranke *Radiologie* 2 (1967) 309
- LUKES R J BUTLER J J and HILKS E H Natural history of Hodgkin's disease as related to its pathologic picture *Cancer* 19 (1966) 317
- NEWALL J The management of Hodgkin's disease *Clin Radiol* 1965 1 p 40
- NICE C M and STENSTROM K W Irradiation therapy in Hodgkin's disease *Radiology* 67 (1954) 641
- — Irradiation therapy in Hodgkin's disease of the thorax *Dis Chest* 28 (1955) 529
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- PATERSON R and PATERSON E Hodgkin's disease *Brit J Med* 2 (1951) 1315
- PETERS M V and MIDDLEMISS K C H A study of Hodgkin's disease treated by irradiation *Amer J Roentgenol* 79 (1958) 114
- PITCOCK J A BAUER W C and MCCABRAN M H Hodgkin's disease in children *Cancer* 12 (1959) 104
- PUSEY W A Cases of sarcoma and of Hodgkin's disease treated by exposures to X rays A preliminary report *J Amer med As* 38 (1902) 166
- SALZMAN F A SWEZAR M I WRIGHT K A and TRUMP J G T₁₀ MeV wide field irradiation of lymphoma *Amer J Roentgenol* 92 (1964) 124
- SAMBROOK D K Clinical aspects of fractionation and dose time relationships *Brit J Radiol* 36 (1963) 174
- SCARFON P W Initial experience with split-dose periodic radiation therapy *Amer J Roentgenol* 84 (1960) 632
- Split-dose radiotherapy Follow up in 50 cases *Amer J Roentgenol* 90 (1963) 280
- SCOTT R M Time-dose relationship in Hodgkin's disease *Radiology* 76 (1961) 276
- and BRIZEL H E Time-dose relationship in Hodgkin's disease *Radiology* 82 (1964) 1043
- SEYDEL H C BROEDORN F G and WITZBERG M J Time dose volume relationship in Hodgkin's disease *Radiology* 89 (1967) 919
- STRANDQVIST M Studien über die kumulative Wirkung der Röntgenstrahlen bei Fraktionierung *Acta radiol* (1944) Suppl No 55
- VAETH J M Pre-treatment of mediastinal Hodgkin's disease *In Progress in radiation therapy* Volume 2 p 164 Edited by F Buschke Grune & Stratton New York and London 1962
- WESTLING P Studies of the prognosis in Hodgkin's disease *Acta radiol* (1965) Suppl No 215

DOSIMETRIC STUDIES OF MANTLE FIELDS IN COBALT 60 THERAPY OF MALIGNANT LYMPHOMAS

by

GUDLA SÄHN-TAPIER

During the past few years an increasing number of radiotherapists have advocated a particular type of radiation treatment for certain forms of lymph node diseases, a significantly larger portion of the body being subjected to radiation than was previously customary. These conditions which were formerly regarded as generalized are now considered to be initially local and therefore possibly curable (CRAVER 1954, KAPLAN 1962, 1966, LARSSON & RUSSEL 1963, PETERS 1966 and MUSHOFF & BOUTIS 1968). It has also been demonstrated that the spread is often at first to the adjacent lymph node groups (JELLIFFE 1965, NEWALL 1965, KAPLAN 1966, ROSENBERG & KAPLAN 1966, LANDBERG & LARSSON 1968, LANDBERG 1969). The best results of treatment should therefore be attained if the clinically involved lymph node groups as well as adjacent groups are irradiated. The submandibular, cervical, supraclavicular, infraclavicular, axillary and mediastinal lymph node groups are consequently all irradiated in supradiaphragmatic conditions. The simplest technique for such treatment is the use of two large opposing anterior and posterior fields with lead blocks interposed to shield tissues not to be irradiated. Large source to skin distances are necessary and the treatment fields are irregular in shape.

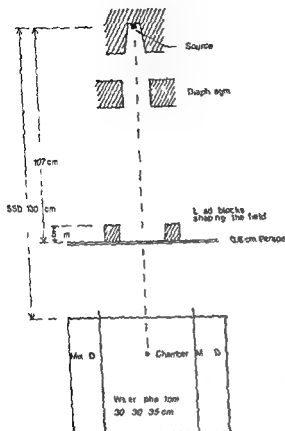


Fig 1 Schematic drawing of apparatus for the determination of depth doses in different parts of the mantle field. The clinical situation is simulated by exchanging the water phantom and mix D blocks for the thorax phantom.

This report describes isodose charts for a sagittal plane in the centre of the field with and without a beam flattening filter and for transverse planes at the levels of the jugulum and the pulmonary hila. A comparison between planned dose distribution and measured dose at certain points in a thorax phantom is also presented.

Material and Methods

Experimental equipment The measurements were carried out with a Siemens Gammatron III which has a cobalt 60 source with a diameter of 1.5 cm. The cobalt 60 unit is equipped with a block diaphragm, its anterior part being 23.8 cm from the source. The edge of the light beam was adjusted to match the 50% isodose line at 0.5 cm depth for a field size of 10 cm \times 10 cm. All

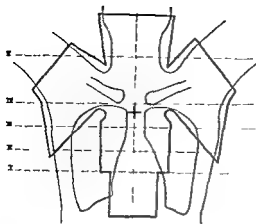
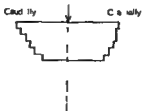


Fig 2 Mantle field with reference to thorax phantom. Dotted lines indicate planes where dose planning and measurements are carried out

Beam flattening filter A



Beam flattening filter B

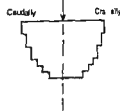


Fig 3 The two beam flattening filters A and B are made of perspex with a density of 1.18 kg/cm^3 source filter distance 30.2 cm

measurements were carried out at an SSD of 130 cm . The beam direction was vertical.

Phantoms used in the measurements All depth dose measurements were performed in a $30 \text{ cm} \times 30 \text{ cm} \times 35 \text{ cm}$ water phantom, and with mix D as side scattering material where the phantom was not sufficiently large. The film exposure for dose determination was made in a polystyrene phantom $35 \text{ cm} \times 35 \text{ cm} \times 30 \text{ cm}$, also with mix D as side scattering material. The thorax phantom consisted of mix D and contained a skeleton, the arms being above the head with the neck extended. The lungs were filled with sawdust to a density of 0.25 g/cm^3 , which according to DAHL & VINTERLOF (1960) is the radiation equivalent of an air filled lung. Unfortunately, the chest wall of the phantom was too thick and the apices of the lungs terminated at the jugulum instead of at Th2.

Beam flattening filters The beam flattening filters used in the cranial-caudal direction were made up of perspex plates, 0.5 cm thick, with a density of 1.18 g/cm^3 . The source filter distance was 30.2 cm (see Fig 3).

Shape and size of the mantle field The mantle field was formed of 5 cm lead blocks placed on a 0.8 cm perspex plate 107 cm from the cobalt source (Fig 1). The sides of the lead blocks were vertical and the shape and size of the mantle field were adjusted to simulate a mantle treatment, the size of the field before the lead blocks were interposed was $38 \text{ cm} \times 40 \text{ cm}$. The mantle

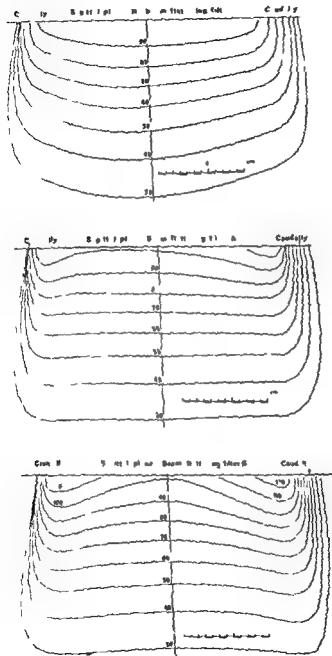


Fig. 4. Isocontour plots for the sagittal plane in the middle of the mantle field, without beam flattening filter (upper) and respectively with beam flattening filter A (middle) and filter B (lower).

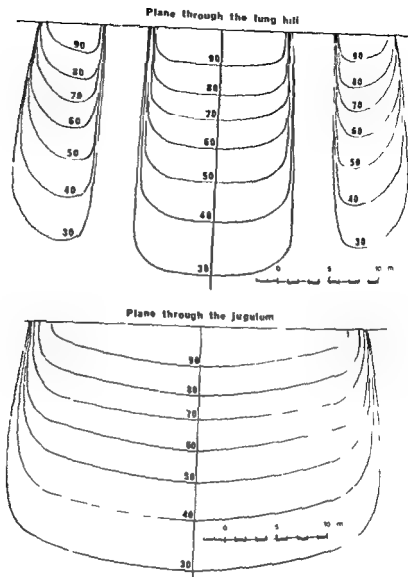


Fig 5 Isodose charts for transverse planes according to fig 9 no beam flattening filter in either direction

field was so formed (Fig 2) that the light beam indicated a border of 1 cm outside the target area, projected vertically onto the surface of the phantom where the target area included all supradiaphragmal lymph nodes from the mastoid process to Th12. The primary field extension outside the target was 3 cm, 2 cm of which were shielded by lead on the cranial and lateral borders. The broad penumbra was left undiminished on the caudal border to facilitate the addition of abdominal fields. With depth dose measurements for square fields, the size of the field was defined by the diaphragm of the cobalt unit.

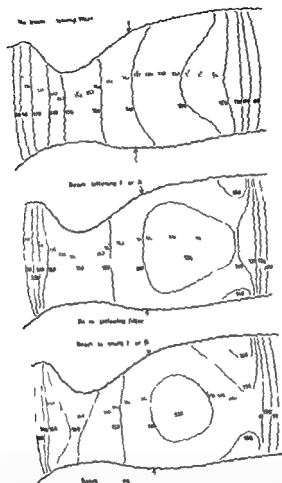


Fig. 6 Dose planning for anterior and posterior fields in the sagittal plane through the middle of the phantom respectively without and with beam flattening filter. \ indicates dose value measured with condenser chambers. The trachea is filled with paraffin pellets.

Measuring procedure Depth dose measurements for all the fields were made with the aid of a cable connected ionization chamber the air volume of which was 1.0 cm³. An ionization chamber of air volume 0.1 cm³ cable connected was also used in the determination of depth doses for field sizes 10 cm \times 10 cm and 17 cm \times 17 cm. All the ionization chambers were calibrated free in air against a substandard chamber which had in turn been calibrated at the Standard Laboratory of the Swedish Radiation Protection Institute. When the ionization

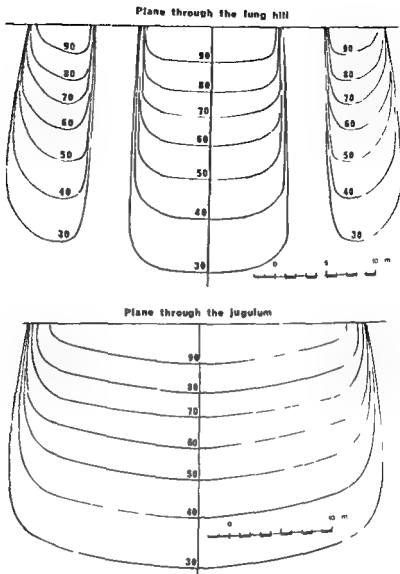


Fig. 1 Isodose charts for transverse plane according to fig. 2 no beam flattening, filter in either direction

field was so formed (Fig. 2) that the light beam indicated a border of 1 cm outside the target area projected vertically onto the surface of the phantom where the target area included all supradaphragmal lymph nodes from the mastoid process to Th12. The primary field extension outside the target was 3 cm, 2 cm of which were shielded by lead on the cranial and lateral borders. The broad penumbra was left undiminished on the caudal border to facilitate the addition of abdominal fields. With depth dose measurements for square fields, the size of the field was defined by the diaphragm of the cobalt unit.

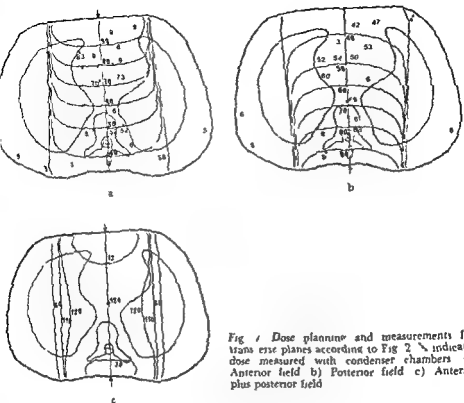


Fig 1 Dose planning and measurements for transverse planes according to Fig 2 'x' indicates dose measured with condenser chambers a) Anterior field b) Posterior field c) Anterior plus posterior field

Results and Discussion

Calculation of depth dose The depth dose for SSD = 130 cm has been calculated for field sizes 0 cm × 10 cm × 10 cm, 17 cm × 17 cm and 20 cm × 20 cm according to the British Journal of Radiology (1961), Suppl No 10. The tissue/air ratio measured by GUPTA & CUNNINGHAM (1966) was used in the calculation. Depth dose calculations for the mantle field were made according to the method of CLARKSON (1941). The field was then divided into sectors of 5°. The scatter function according to GUPTA & CUNNINGHAM (1966), is given as

$$S(r_x, d, F) = 100 \left(\frac{F+x}{F+d} \right)^2 [T(r_x, d) - \exp\{-\mu(d-x)\}]$$

where the scatter function is defined as the dose due to scattered radiation at points along the central axis of the beam per 100 rad from primary radiation

chambers are used for dose measurements at different depths in a phantom the photon spectrum differs considerably from the spectrum free in air, and the secondary radiation is almost isotropically incident on the ionization chamber. Photon spectra for different depths and for different field sizes are reproduced in the diagram of BRUCE & JONES (1960).

According to calculations, the equivalent area to the mantle field is approximately $25\text{ cm} \times 25\text{ cm}$. The calibration constant for the ionization chamber of volume 10 cm^3 determined free in air varied less than 2% for HVL 0.2 mm Cu to HVL 14.6 mm Cu. Thus, no corrections for energy dependence were made and the possible direction dependence for cobalt radiation could be ignored. In measurements with the ionization chamber of volume 0.1 cm^3 these two factors were eliminated through a comparative measurement using the same field size at a SSD of 100 cm for which depth doses are known (Brit J Radiol 1961). The difference in the depth dose measurements of the small and large ionization chambers was 2% of the measured value for a depth of 20 cm, after standardizing to 5 cm depth. Depth dose measurements in water for mantle fields were also carried out by means of a Riecke dosimeter. The volume of the dosimeter was 4 ml.

Depth dose measurements made in eight different radiation directions, distributed evenly over the mantle field, form the basis for the determination of isodose charts. Measurements were carried out with the aid of the cable connected ionization chamber of volume 10 cm^3 . Supplementary measurements were made with condenser chambers of volume 0.3 cm^3 at depths of 5 cm and 15 cm. Film dosimetry was used for further help in drawing the isodose lines. It proved to be impossible to obtain a linear relationship between film density and exposure within the whole mantle field, since the energy spectrum differs between the central and distal parts of the field. Isodensity lines were therefore used only for the distal parts of the field, where an explicit relationship between density and exposure could be obtained. In the other parts of the mantle field they were used only as an aid in drawing the isodose lines between the dose values measured by the ionization chamber.

The thorax phantom was dose planned centrally in a sagittal plane both with and without beam flattening filter as shown in Fig. 6, and in five transverse planes as seen in Figs 7—11. Correction for irregular body contour was made with the isodose shift method (DUTREIX & DUTREIX 1962). In the axillary and submandibular regions the isodoses were shifted in the direction of the divergence of the local beam. The dose measurements in these transverse planes as well as in the oesophagus were carried out with the aid of condenser chambers.

All figures in the isodose charts and dose planes are written on the higher dose side of the isodose line.

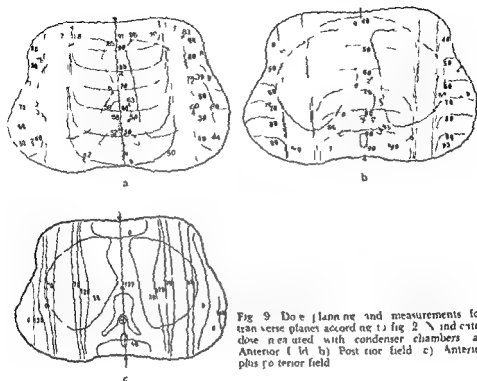


Fig 9 Dose planning and measurements for transverse planes according to fig 2. Indicates dose measured with condenser chambers a) Anterior field b) Posterior field c) Anterior plus posterior field

For the point below the centre the calculated dose at a depth of 10 cm is 62.5% and the dose measured with the ionization chamber 61.8%. If the field is 4 cm longer in a caudal direction the calculated dose at 10 cm is 63.1% and if the field is shortened 4 cm caudally the calculated dose becomes 61.3%.

Comparison between calculated and measured depth dose A comparison is made in Table 1 between measured and calculated values of depth doses, the former being standardized to those calculated at 5 cm. The mean values of measurements with two ionization chambers are entered in the table for the fields 10 cm \times 10 cm and 17 cm \times 17 cm. The uncertainty in the Fricke measurements at a depth of 14.6 cm is $\pm 5\%$ of the depth dose value. The difference in measured and calculated values for greater depths may possibly depend on the difference in the back scattering material. At 20 cm depth 10.7 cm of phantom material lay behind the ionization chamber while the tissue/air ratios and scatter functions were determined in a water phantom that was 60

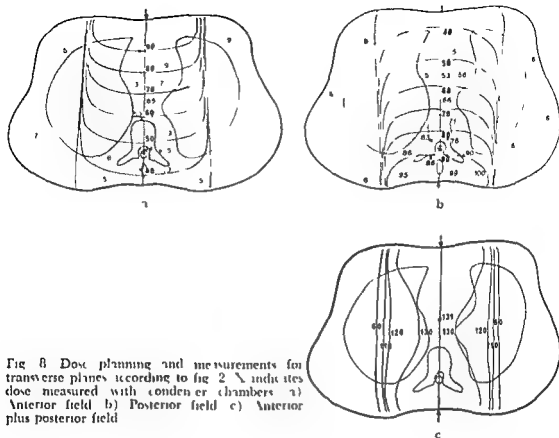


Fig 8 Dose planning and measurements for transverse planes according to fig 2 λ indicates dose measured with condenser chambers a) Anterior field b) Posterior field c) Anterior plus posterior field

alone at the position of the maximum dose in the phantom, λ is the depth of maximum dose, r_x the radius a circular beam would have at depth λ . F is the SSD, $T(r, d)$ is the tissue/air ratio for depth d in the phantom and μ is the narrow beam attenuation coefficient for the phantom material

Since the tissue/air ratio is independent of SSD $S(r, d, 130)$ may be written

$$S(r, d, 130) = \frac{\left(\frac{130-5}{130+d}\right)^2}{\left(\frac{80-5}{80+d}\right)^2} S(r, d, 80)$$

$$\text{where } r_x = \frac{80-5}{80+d} \frac{130+d}{130-5} r$$

Scatter functions for SSD = 80 cm have been tabulated by the same authors. Calculation of the depth dose is made for the centre of the field (Table 1) and for a point 11.6 cm below the centre. The scatter function for the mantle field has the same value as for a square of 25 cm \times 25 cm

Table 1

Calculated and measured percentage depth doses for SSD = 130 cm. The measured depth doses are standardized to the calculated doses at 5 cm

Depth cm	Field size						Mantle field equivalent square 25 cm 25 cm		
	10 cm	10 cm	15 cm	17 cm	20 cm x 20 cm	20 cm x 20 cm	Calculated	Measured	Measured
	Calculated	Measured in chamber	Calculated	Measured in chamber	Calculated	Measured in chamber	Calculated	Measured in chamber	Measured Fricke dosimeter
0.5	100	99.6	100	98.9	100	98.3	100	99.2	
1.0								99.2	
2.0	94.9		90.5		90.7				
3.0	81.9	81.9	83.9	83.9	81.4	81.4	84.8	84.8	84.9
4.0							67.4		67.4
10.0	60.3	60.3	64.1	64.4	60.7	60.3	66.6	67.0	
14.6							51.7		52.5
15.0	43.4	44.4	47.9	49.1	49.2	49.4	51.1	50.1	
20.0	31	30.1	32.8	36.4	37.2	36.8	39.1	37.7	

Table 2

Comparison of the dose rates in the central line of mantle fields of different sizes

Depth cm	Field including the phantom including hila	Field including the phantom excluding hila	Field including a small patient in- cluding hila	Field including a tall patient in- cluding hila
1	100	98.5	98.9	101
2	10	49.6	50.0	52.0

has a greater antero-posterior thickness in the submandibular and mediastinal regions as compared to the jugulum level the filters are overcompensating to produce a homogeneous dose as possible to the whole target volume. The depth dose at less than 20 cm depth in the centre of the field with beam flattening filters does not differ from the depth dose without filter.

Comparison of planned and measured dose in a thorax phantom. The upper charts a and b in Figs 7, 8, 9 and 10 indicate the planned and the measured dose values respectively for both the anterior and the posterior treatment field in the transverse planes I to IV as in Fig 2. Measurements and dose

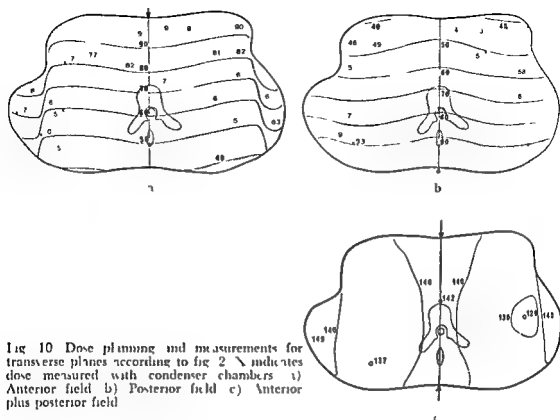


Fig 10 Dose planning and measurements for transverse planes according to fig 2 \downarrow indicates dose measured with condenser chambers a) Anterior field b) Posterior field c) Anterior plus posterior field

cm deep. The measured values have been used in the isodose charts but standardized to 100 % at 0.5 cm depth.

Dose rate variation according to the shape and size of the mantle fields The mantle field must be modified individually in the treatment of patients and therefore the shape and size may differ from one patient to another. The measurements were made in a water phantom representing two extreme fields, the one in a child of 12 years (157 cm, 41 kg) and the other in a male adult (190 cm, 100 kg). The measurements were carried out in the centre, and the back scatter material was the same for all fields. A comparative measurement in which the hilar region was covered with lead blocks was also performed. The results are presented in Table 2.

Isodose charts Isodose charts centrally in a sagittal plane are shown in Fig 4, and for transverse planes at respectively the hilar and jugulum levels in Fig 5. The depth dose in the transverse planes was adjusted to the depth dose in the sagittal plane at the corresponding level. Two beam flattening filters (cf Fig 3) were made for the sagittal plane. Since the patient always

Table 1

Calculated and measured percentage depth doses for SSD = 130 cm — The measured depth doses are standardized to the calculated doses at 5 cm

Depth in cm	Field sizes		Mantle field equivalent in square 25 cm x 25 cm					
	10 cm x 10 cm	17 cm x 17 cm	20 cm x 20 cm					
	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber
0.5	100	98.6	100	98.9	100	98.5	100	99.9
1.0								99.2
2.0	94.9		93.5		93.7			
3.0	81.9	81.4	83.4	83.9	83.4	83.4	84.8	84.8
3.8							67.4	67.4
10.0	60.3	60.5	64.1	64.4	63.2	63.3	66.6	67.0
14.1							51.7	51.5
15.0	43.4	44.4	47.9	49.1	49.2	49.4	51.1	50.6
20.0	31.2	30.7	35.8	36.4	37.9	36.8	39.1	37.7

Table 2

Comparison of the dose rates in the central line of mantle fields of different sizes

Depth cm	Field using the phantom including hula	Field using the phantom including hula	Field using a small patient including hula	Field using a tall patient including hula
1.7	100 51.0	93.5 49.6	98.9 50.0	101.9 52.0

has a greater antero posterior thickness in the submandibular and mediastinal regions as compared to the jugulum level the filters are overcompensating to produce as homogeneous a dose as possible to the whole target volume. The depth dose at less than 20 cm depth in the centre of the field with beam flattening filters does not differ from the depth dose without filter.

Comparison of planned and measured dose in a thorax phantom The upper charts (a and b) in Figs 7, 8, 9 and 10 indicate the planned and the measured dose values respectively for both the anterior and the posterior treatment fields in the transverse planes I to IV as in Fig. 2. Measurements and dose

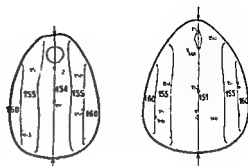


Fig 11 Dose planning and measurements for two phantoms of the neck differing in size λ indicates the dose measured with condenser chambers Air in trachea

planning were carried out for two different phantoms of the neck, since the neck contour of patients often differs considerably (Fig 11). The trachea was filled with paraffin pellets in the oesophagus measurements (Fig 6). Measurements made with air in the trachea, where the diameter of the air cavity was 16 mm, produced a 7% increase in the hypopharynx and the oesophagus versus the subcutaneous maximum dose in the centre of the ventral field. Thus to obtain the best possible interpretation of the measured values in the hypopharynx and the oesophagus the position of the catheter with ionizing chambers in relation to the trachea, should always be determined with the aid of a film exposed during the treatment.

A statistical analysis of the accuracy of the measured values is difficult to carry out since systematic errors are introduced. For example, no correction was made for inhomogeneous tissue and the correction for the body contour was made with the isodose shift method, even when the angle of incidence was more than 45° . No consideration was made for the smaller mass of the side scattering material in the thorax phantom measurements as compared with the water phantom measurements. The accuracy of the ionization chamber

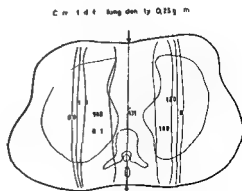


Fig 12 Dose planning in the hilar region for anterior and posterior fields with correction for lung density 0.25 g/cm^3 (Cf fig 8c)

measurements is $\pm 4\%$ of the measured value this figure includes an energy dependence of about 1%

Lung correction at the hilar level To calculate the lung correction the ionization chamber measurements were performed for the anterior field at four different lung depths at the hilar level. If the depth dose curve in water for the mentioned level in the mantle fields is drawn as a straight line in a semi logarithmic diagram for depths greater than 5 cm the measurements in the lung plotted in the same diagram follow a line with a slope of 0.6 compared with the depth dose decrease in water.

The isodose distribution at the hilar level with lung correction is indicated in Fig. 12 (cf. Fig. 8c).

Conclusion

In spite of all uncertainties introduced in the treatment planning of the thorax phantom the agreement with the dose measured at certain points is surprisingly good. In the mantle treatment of patients it should therefore be sufficient to control the dose in the sagittal plane by measurements in the hypopharynx and oesophagus for checking the effect of the field size, the patient's size and the possible movements of viscera between the two treatment positions.

To obtain as homogeneous a dose as possible in the whole target area a beam flattening filter should be used in a cranial-caudal direction with an individual filter in the cervical region. The hilar regions of the lungs should be shielded with lead during some of the treatments to prevent a too high dose to the lungs.

SUMMARY

The dosimetry for mantle fields in the treatment of supradiaphragmatic malignant lymphomas with cobalt 60 was analysed. Isodose charts for the mantle field were constructed in sagittal and in different transverse planes. Studies of the agreement between planned and measured doses in a thorax phantom were performed both with and without beam flattening filters.

ZUSAMMENFASSUNG

Die dosimetrischen Grundlagen für Mantelfelder wie diese bei der Behandlung von supradiaphragmatischen malignen Lymphomen mittels Cobalt 60 herkömmlich sind wurden studiert. Es wurden Isodosenpläne für Mantelfelder in der Sagittalebene und in verschiedenen Transversalebene konstruiert. An einem Thoraxphantom wurde das Übereinstimmen der geplanten Dosis mit der wirklich gemessenen Dosis mit und ohne dem Gebrauch von Ausgleichfiltern geprüft.

RÉSUMÉ

L'auteur a analysé la dosimétrie des champs en mantelet pour le traitement des lymphomes malins des diaphragmatiques par le cobalt 60. Elle a construit des courbes isodoses pour le champ en mantelet dans un plan sagittal et dans différents plans transversaux. Elle a étudié la concordance entre les doses prévues et les doses mesurées sur un fantôme du thorax avec et sans filtre compensateur du fusceau.

REFERENCES

- BRUCE W. R. and JOHNS H. F. The spectra of X rays scattered in low atomic number materials. *Brit J Radiol* (1960) Suppl No 9.
- CLARKSON J. R. A note on depth doses in fields of irregular shape. *Brit J Radiol* 14 (1941) 265.
- CRABER L. I. Some aspects of the treatment of Hodgkin's disease. *Cancer* 7 (1954) 927.
- DAMIL O. and VIKTORIOF K. J. Attainment and value of precision in deep radiotherapy. *Acta radiol* (1960) Suppl No 189.
- DUPUIS DOSE TABLES FOR USE IN RADIOTHERAPY. *Brit J Radiol* (1961) Suppl No 10.
- DUTREIX A. et DUTREIX J. Construction des isodoses pour les surfaces obliques et irrégulières. *J Radiol Electrol* 13 (1962) 671.
- LASSON F. C. and RUSSELL M. H. The cure of Hodgkin's disease. *Brit med J* 1963 1 p 1704.
- GUPTA S. K. and CUNNINGHAM J. R. Measurement of tissue air ratios and scatter functions for large field sizes for cobalt 60 gamma radiation. *Brit J Radiol* 39 (1966) 7.
- JILLIFFE A. M. The present place of radiotherapy in the cure of Hodgkin's disease. *Clin Radiol* 16 (1965) 274.
- KAPLAN H. S. The radical radiotherapy of regionally localized Hodgkin's disease. *Radiology* 78 (1962) 553.
- Role of intensive radiotherapy in the management of Hodgkin's disease. *Cancer* 19 (1966) 356.
- LANDBERG I. Clinical course of Hodgkin's disease treated with radiotherapy. *Acta radiol Ther Phys Biol* 11 (1969) 487.
- und LARSSON I. G. Studium des klinischen Verlaufs bei Steinberg'scher Erkrankung. *Radiol Austr* 18 (1968) 197.
- MUSCHOFF K. and BOUTIS L. Therapy results in Hodgkin's disease. *Freiburg im Breisgau* 1948—1966. *Cancer* 21 (1968) 1100.
- NEWALL J. The management of Hodgkin's disease. *Clin Radiol* 1 (1965) 40.
- PETERS M. V. Prophylactic treatment of adjacent areas in Hodgkin's disease. *Cancer Res* 26 (1966) 1232.
- ROSENBLERG S. A. and KALLIAN H. S. Evidence for an orderly progression in the spread of Hodgkin's disease. *Cancer Res* 26 (1966) 1225.

EFFECT OF SELECTIVE TUMOR HEATING ON THE LOCALIZATION OF ^{125}I FIBRINOGEN IN THE WALKER CARCINOMA 256

I Heating by immersion in warm water

by

EDMUND S. COPELAND

Intravenously administered ^{125}I labeled fibrinogen or antibody to fibrin localizes preferentially enough in some transplantable rat tumors that it can be used for effective tumor radiation therapy (DAY et coll 1959 BALE et coll 1960). Localization also occurs quite commonly in human tumors, sometimes to an extent that the use of this method for therapy seems feasible. However attempts at therapy have not produced prolonged remissions (McCARDLE et coll 1966). In many human tumors and in some transplantable rat tumors, in particular the Walker carcinoma 256 the localization of radioiodinated fibrinogen is not remarkable.

The purpose of the present work is to investigate methods by which the localization of ^{125}I fibrinogen in the Walker carcinoma 256 may be enhanced with the hope that successful techniques might serve as useful adjuncts to human cancer therapy with radioiodinated fibrinogen. It has been suggested that deposition of fibrin results from the invasive growth of the tumor and is due perhaps to

an inflammatory reaction of the host tissues or to simple injury of blood vessels of supporting stroma or invaded tissues (BAIR et coll 1960)

In this laboratory, the localization of ^{125}I fibrinogen in two transplantable rat tumors has been studied extensively. In the course of this investigation, localization will refer to the amount of ^{125}I activity found in a specific site and presumably represents the result of a steady deposition and destruction of fibrin. SHAEFFER (1962) has shown that about 20 % of the injected radioactivity localizes in a 2.5 gram Murphy Storm lymphosarcoma 18 hours after intravenous administration of ^{125}I fibrinogen. A 3 gram Walker tumor, on the other hand, as reported later, localizes only 1 % of the injected dose 3 days after ^{125}I fibrinogen injection. If the percentages of the remaining whole body activity found in the tumors at these times are calculated for these two tumors, one obtains 30 % and 8 % for the Murphy Storm and Walker tumors, respectively. According to HIRAMOTO et coll (1960), the small amount of localization in the Walker tumor might be explained in part on the basis of its slower growth rate as compared to the Murphy tumor. However, studies by SOVARDI (1959) suggest that rats bearing the Walker tumor have increased plasma fibrinolytic activity and slightly reduced blood coagulability. VARON & SPAR (1965) have demonstrated that enhancement of the coagulation mechanism of the host increases fibrinogen localization in the Murphy Storm lymphosarcoma. BAIR et coll (1962) have shown that when rats bearing the Walker tumor are given epsilon aminocaproic acid (EACA) in their drinking water, a marked increase in tumor fibrin localization occurs. MUTSCHLER (1963, 1964) has extensively investigated the effect of EACA as a fibrinolysis inhibitor. Her results were corroborated in this investigation and are discussed in detail below.

A simple model can serve as the basis for experimentation: tumor fibrin localization equals depositions minus removal. Its removal from the tumor site involves fibrinolytic processes, thus systemic inhibition of fibrinolysis should result in increased tumor fibrin localization. Tumor fibrin deposition may be hypothetically divided into two phases: fibrinogen extravasation due to a localized inflammatory reaction or to invasive damage to vasculature, and fibrinogen transformation to fibrin by the coagulation mechanism. It seems reasonable then that at least three means could be employed to increase the amount of fibrin localized in the Walker and other tumors: (1) inhibition of the fibrinolytic system of the host; (2) enhancement of any inflammatory reaction occurring at the tumor site, and (3) selective damage to tumor tissue.

If tumor cells and cells of the tumor stroma, such as mast cells and capillary endothelial cells, could be selectively damaged in some way, mediators of the inflammatory reaction such as histamine might be released at the site of the insult and increased fibrinogen extravasation would occur. If tissue thromoplastin

were also released from the damaged cells this extravasated fibrinogen should clot at the site of injury. It is also possible that selective tumor damage might directly injure the vasculature of the supporting stroma and in this way increase fibrinogen extravasation.

In 1935 WARREN studied the effects of artificially induced fever upon hepatic tumor cases. Infrared heat lamps were used to induce fever (rectal temperature $+1.5^{\circ}\text{C}$) for periods up to 21 hours. After such treatment tumor growth was temporarily retarded and the patient's life was prolonged. His work shows that tumor tissue is more susceptible to destruction by heat than are the tissues in which it grows. CRILE JR (1963) has reported that heating certain melanomas implanted in the feet of mice to 44°C for from 30 to 40 minutes destroyed a high proportion of the tumors without damage to the feet.

Materials and Methods

Rat fibrinogen. Fraction 1 was obtained from fresh oxalated rat plasma by alcohol addition in the cold (ice salt bath) according to method 6 of COHN et coll (1946) modified by the omission of acetate buffer. The procedure is essentially that reported by SHAEFFER (1964).

Labeling of fibrinogen with ^{125}I . The protein iodination method of HELM KA et coll (1960) using iodine monochloride as modified by BALE et coll (1966) to include the use of catalase to eliminate H_2O_2 was followed throughout the studies.

^{125}I labeled rat albumin. Rat albumin was isolated from fresh rat serum according to a modified version of the acidified methanol technique of MICHAEL (1962). This twice recrystallized albumin was then radioiodinated using the technique described for fibrinogen iodination. The purity of the ^{125}I albumin preparation was then tested by several methods. Only 1.5% of the radioactivity of an aliquot was bound to proteins precipitable with thrombin. More than 97% of the radioactivity of an aliquot precipitated in 20% trichloroacetic acid. Nearly 93% of the radioactivity of ^{125}I albumin spiked plasma or serum samples remained in solution when one third volume saturated ammonium sulfate was added. Electrophoresis studies indicated that not more than 5% of the sample radioactivity could be fibrinogen bound.

Experimental animals. Sprague Dawley female rats (Holtzmann Farms Wisconsin) weighing 100–200 grams each were used. Potassium iodide drinking water ($6 \times 10^{-4}\text{M}$) was given to all experimental animals to reduce the uptake of inorganic ^{125}I by the thyroid. Animals were given checkers of Purina Labora-

tory Chow (for rats) *ad libitum*. Two rats were housed per cage. In all experiments in which LACA was employed, a 5% solution of LACA in the drinking water was given to the rats at least 18 hours prior to fibrinogen injection.

Walker carcinoma 256 A line of Walker carcinoma 256 (JARIE 1935, FISHER & FISHER 1961) which for some years has been carried in this laboratory and was originally obtained from Dr Florence Miller, National Institutes of Health, Bethesda, Maryland, was transplanted by trocar into Sprague Dawley rats. A single fragment (about 15 mg.) from a vascular non necrotic tumor area was placed subcutaneously on the right side approximately opposite the lowest rib. The transplant site had a barely palpable tumor on the seventh day after inoculation, which grew to a 10 to 14 gram tumor in six more days.

¹³¹I fibrinogen injection and radioactivity measurements Immediately prior to its injection, the previously iodinated frozen fibrinogen was thawed and diluted with fresh oxalated rat plasma to an activity concentration of about 200 μ Ci per ml. Diluted fibrinogen was assayed by a clotability test within 2 hours prior to injection and was found to contain 82 to 92% clottable protein. Diluted fibrinogen solution was injected via the sphenous vein in a volume of 0.5 ml. Immediately after the injection a whole body radioactivity measurement was made by using MITSCHNER'S technique (1963, 1964). Counts were compared to those from an injection standard prepared by adding 0.5 ml of injection solution to 100 ml of distilled water made basic with several NaOH pellets in a 250 ml flask. (The standard was made basic to keep any non protein bound ¹³¹I in the reduced non vaporizable iodide form.) Additional whole body counts were made prior to sacrifice. Immediately after sacrifice in ether, the rats were weighed. The tumor was then carefully removed, weighed, and counted. The remainder of the animal (the carcass) was similarly counted.

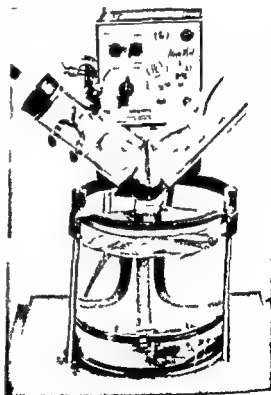


Fig. 1 The tumor heating apparatus

The remainder of the animal (the carcass) was similarly counted.

Tumor heating technique For selective tumor heating a Blue M Magna Whirl Jar Bath (Blue M Electric Company Blue Island, Illinois) was used. In order to heat the tumor alone, the rat was placed in an L shaped lucite holder which had a one inch diameter hole in the outside wall of the angle through which the subcutaneous tumor could be pulled. The tumor was then pulled through a small hole in a thin rubber sheet placed outside the lucite container to prevent entrance of warm water (see Fig. 1).

In order to keep the rats immobile during treatment, they were anesthetized with a fluothane-oxygen anesthetic. Fluothane the Ayerst Laboratories brand of halothane was kindly supplied by Dr J. H. Jewell of Ayerst Laboratories 685 Third Avenue New York New York 10017. Oxygen loaded with fluothane was allowed to enter the anesthesia chamber at 0.2 cubic feet per hour and 100% oxygen entered the chamber at 2.0 cubic feet per hour.

Radioautography studies For radioautographic and further histologic study tumors were fixed in a buffered 10% formalin solution exposed to no-screen roentgen film as paraffin sections and later stained with hematoxylin and eosin. The film was exposed for 48 to 96 hours depending on the activity of the ^{131}I in the sections.

Experimental results

Quantitative evaluation of the effect of the various treatments required parameters measuring ^{131}I localization. The parameters used in this study are as follows:

Percentage of injected dose per gram tumor normalized ($\% \text{ID/g TN}$) This parameter thus involves a normalization of localized radioactivity to correct for the differences in body weights of the individual experimental rats. The parameter is calculated as follows:

$$\% \text{ID/g TN} = \frac{\text{net counts per min from tumor} \times \text{whole body weight}}{\text{net counts per min from injection standard} \times \text{tumor wt}}$$

Therapeutic ratio The value of radiation treatment of a cancerous growth is usually judged according to the magnitude of the ratio

$$\frac{\text{rad absorbed by malignant tissue}}{\text{rad absorbed by normal tissue}}$$

Table I

Effect of mechanical traumatization on tumor fibrinogen localization

Group	Number of rats	% ID/g TN	Therapeutic ratio	3 day whole body radioactivity retained (%)
No treatment	28	1.06 ± 0.07	7.9 ± 0.7	19.5 ± 1.1
Daily mechanical traumatization	"	2.36 ± 0.42*	22.2 ± 5.2*	30.4 ± 4.1*

* Indicates a statistically significant difference from the control value at the 1% significance level. In this and following table each parameter is followed by ± standard error of the mean.

This ratio can be approximated for the case of a rat bearing the Walker tumor and having been administered a certain injected dose of ^{131}I fibrinogen

$$\frac{\text{percentage injected dose per gram tumor tissue}}{\text{percentage injected dose per gram normal tissue}} = \frac{\% \text{ ID/g tumor}}{\% \text{ ID/g carcass}}$$

This latter approximate calculation will be referred to as the therapeutic ratio for the purposes of this study.

Percentage of whole body radioactivity retained. If one animal of an experimental group had a radioactivity retention which was greater than two standard deviation units higher than the mean of the group, it was usually an indication that some of the ^{131}I fibrinogen had been injected into the tissue surrounding the saphenous vein rather than intravenously and was clotted there under the action of tissue thromboplastin. The data from such experimental animals was discarded. The percentage of whole body retention was calculated as follows:

$$\text{percentage whole body retention on day } X = \frac{\frac{\text{whole body counts on day } X}{\text{standard counts on day } X}}{\frac{\text{whole body counts at injection}}{\text{standard count at injection}}} \times 100$$

Thus, by comparison with an injection standard, the whole body counts on experimental animals were corrected for the physical decay of ^{131}I .

Effect of epsilon aminocaproic acid (EACA) on the localization of ^{131}I fibrinogen in the Walker carcinoma 256. The data now reported were gathered from experiments in which control rats received either KI or EACA plus KI in their drinking water but no additional treatment. In each case, Walker tumor

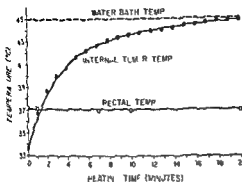


Fig. 2 Effect of selective warm water tumor heating on tumor and rectal temperature

bearing rats were placed on either KI or 5 % EACA KI nine days after tumor transplant and one day prior to ^{131}I injection. After three days the tumor was carefully excised. The 23 control rats which had only KI in their drinking water showed a % ID/g TN of 0.14 ± 0.03 whereas this parameter for the 27 rats drinking 5 % EACA KI was 1.48 ± 0.17 . Thus, an increase of tumor fibrinogen localization by a factor of 3.4 was induced by EACA. This figure is within the range previously reported (BALE et al 1962 MITSCHLER 1963 1964).

Effect of mechanical traumatization on tumor fibrinogen localization. Preliminary experiments indicated that room temperature heating of control tumors caused considerably more tumor fibrinogen localization than nontreatment of controls. That this increased localization could be due to mechanical traumatization during positioning is shown by the results in Table 1.

These results indicate that rough handling of the Walker tumor can lead to marked increase of ^{131}I fibrinogen localization there and that mock treatment of control animals is necessary in order that the effect of tumor heating *per se* can be studied.

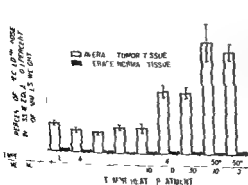


Fig. 3 Effect of warm water tumor heating on localization of ^{131}I fibrinogen in Walker carcinoma 256. The percentage of injected radioactivity dose remaining in tumor and non-tumor portions of rats whose tumors were subjected to heat treatment for the time and temperature indicated is plotted here in the form of a bar graph. Values are expressed as percentage of injected ^{131}I dose in an amount of tissue equal to 1 g of animal's total weight. Vertical line at top of each bar indicates standard error of mean (Standard error of the mean values for average normal tissue were not large enough to be reproduced).

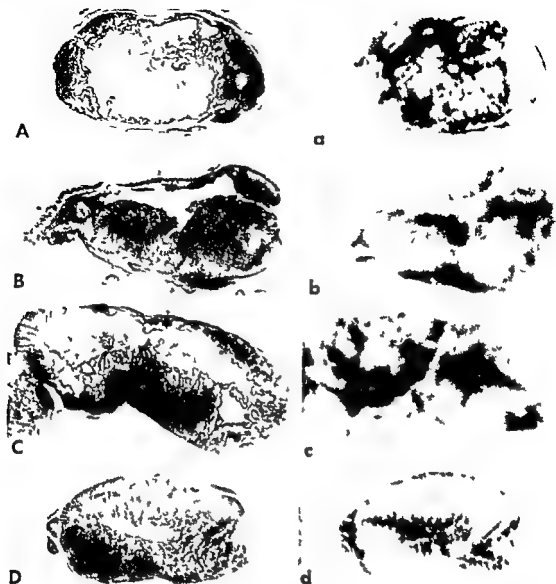


Fig. 4 Photomicrographs and radioautographs from selective tumor heating study (warm water) 3 day uptake magnification $\times 14$

A—a) Control tumor (K1—20) Central area of tumor section composed of light pink cytoplasmic material with low nuclear density and empty space region thought to be necrotic. Outer margins of tumor (cortex) composed of cells with large dark blue nuclei and little cytoplasm. Most radioactivity in transition zone between these two areas.

B—b) Treated tumor (42 C—20) There is a large acellular cavity just below skin surface probably filled with bloody fluid at sacrifice and surrounded by narrow band of predominantly cytoplasmic cells. Tumor surrounded by considerable amount of muscle and connective tissue. Radioactivity in area around cavity and in connective tissue.

C—c) Treated tumor (42 C—40) Tumor is considerably larger than others in the series and has a large lightly stained medulla. Radioactivity in transition zone between medulla and cortex.

D—d) Treated tumor (42 C—30) Tumor similar in structure to tumor in A—a but has a much smaller medulla. Radioactivity in the lightly stained medulla.

Effect of selective tumor heating on the localization of ^{125}I fibrinogen in the Walker carcinoma 256 Actual tumor temperature during a typical heat treatment was measured by a thermistor placed in the center of the tumor with precautions taken to prevent water seepage into the tumor (Fig 2). A rapid temperature rise occurred for about 8 minutes until 43°C was reached, and then the temperature gradually increased 1.8°C in the remaining 12 minutes. The water bath was kept at 45°C and the tumor temperature was monitored for 20 minutes.

Results of a treatment temperature series are compiled in Fig 3. Only in the last four treatment groups was a statistically significant difference from the control values noted in fibrinogen localization.

Representative stained sections and the corresponding radioautographs from each group in the above study are illustrated in Figs 4 and 5. These radioautographs suggest that ^{125}I fibrinogen localization took place in necrotic tumor tissue and that heat treatment increased tumor necrosis.

At least 20 minutes at 45°C was necessary to enhance fibrinogen localization in the tumor. Treatment at 50°C produced too much injury to normal tissue for it to be a feasible means of fibrinogen localization enhancement.

In an additional study the hind legs of several animals were heated at 50°C 10, 50°C 20, 45°C 20, and 45°C 30. The hind legs which were heated at 50°C were badly injured. Hind legs which were heated at 45°C swelled somewhat at the time of treatment but did not show subsequent visible damage. When the parameter $\% \text{ ID/g TN}$ is compared for tumor and hind leg muscle heated at 45°C for 20 minutes, one finds 3.87 ± 0.62 and 0.17 ± 0.05 for tumor and muscle respectively. At this temperature then, tumor tissue is much more susceptible to heat damage than is normal muscle tissue as evidenced by the degree of fibrinogen localization.

Kinetics of tumor fibrinogen localization after heating at 45°C 30 Control and experimental animals were sacrificed at the following times after initiation of the 30 minute heat treatment: 0.5, 2, 4, 2, and 61 hours. ^{125}I fibrinogen was injected intravenously just before beginning heat treatment. The variation of control and experimental therapeutic ratios with time is plotted in Fig 6. This parameter of fibrinogen localization appears to increase exponentially with time in both groups after 20 hours.

In the second kinetics of uptake study, control and experimental rats were injected just before or 24 hours after treatment and sacrificed 24 hours later. The results are tabulated in Table 2. The greatest 24 hour localization took place in the group injected immediately after being treated.

If the two experiments are considered as a whole, several trends become

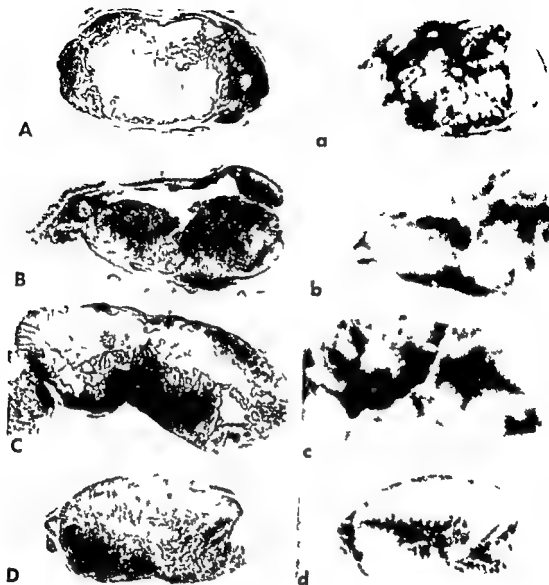


Fig. 4 Photomicrographs and autoradiographs from selective tumor heating study (water) 3 day uptake magnification $\times 14$

A—a) Control tumor (R1—20) Central area of tumor section composed of light pink cytoplasmic material with low nuclear density and empty space region thought to be necrotic. Outer margins of tumor (cortex) composed of cells with large dark blue nuclei and little cytoplasm. Most radioactivity in transition zone between these two areas.

B—b) Treated tumor (42 C—20) There is a large acellular cavity just below skin surface probably filled with bloody fluid at sacrifice and surrounded by narrow band of predominantly cytoplasmic cells. Tumor surrounded by considerable amount of muscle and connective tissue. Radioactivity in area around cavity and in connective tissue.

C—c) Treated tumor (42 C—40) Tumor is considerably larger than others in the series and has a large lightly stained medulla. Radioactivity in transition zone between medulla and cortex.

D—d) Treated tumor (42 C—30) Tumor similar in structure to tumor in A, but has a much smaller medulla. Radioactivity in the lightly stained medulla.

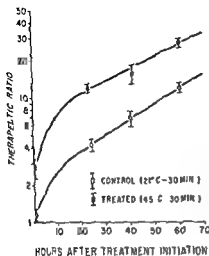


Fig 6 Kinetics of ^{131}I fibrinogen localization in Walker carcinoma 256 after warm water tumor heating at 45°C -30. Therapeutic ratio plotted against time in hours which elapsed between beginning of heat treatment and sacrifice. Vertical bars at each point indicate variation allowed by the standard error of mean. The therapeutic ratios of both the heated and control groups seem to increase exponentially with time after 40 hours.

evident. The greatest amount of radioactive fibrinogen is deposited in the tumor during the first day after treatment. A smaller amount is deposited in the tumor during treatment. The exponential increase of therapeutic ratio with time suggested by Fig 6 probably results from a greater biologic half time of ^{131}I fibrinogen in the tumor than in the remaining body tissues (see Fig 8).

Representative stained tumor sections and the corresponding radioautographs are presented in Fig 7. There did not seem to be any change with time in the pattern of ^{131}I fixation in either the control or heated tumors. In the control tumors radioactivity was found predominately in the center of the tumor in an

Fig 5 (see opposite page) Photomicrographs and radioautographs from selective tumor heating study (warm water) 3 day uptake magnification $\times 17$.

E-e) Treated tumor (45°C -10). First tumor in series to show some effect of heat treatment composed almost entirely of light-staining regions. Radioactivity distributed irregularly throughout this region. Area of highest density composed of cells almost completely devoid of nuclei.

F-f) Treated tumor (45°C -20). Most of this tumor composed of lightly stained areas, cells loosely packed with large nuclei and large cytoplasm surrounded by pink filaments (fibrin). Radioactivity throughout tumor and most dense in the lightly stained areas.

G-g) Treated tumor (45°C -30). Tumor is similar to the one in F-f. It is almost completely composed of loosely packed cells imbedded in filamentous material, entire section lightly stained and radioactivity spread throughout the section.

H-h) Tumor similar to both the two preceding ones. Some erosion of tumor can be seen. There are a small region of normal tumor cells of cortex type which has almost no radioactivity and a long blood-filled cavity which is not especially radioactive. Radioactivity is greatest in area immediately surrounding blood cavity and in the area bordering the cortex type cells.

I-i) Treated tumor (50°C -0). There is considerable tumor erosion. Radioactivity is dense throughout tumor and is minimal in muscle and connective tissue.

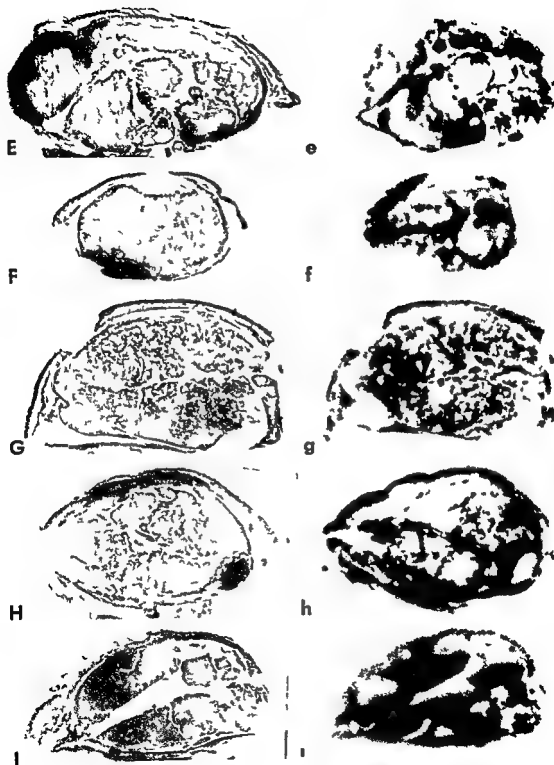


Fig 5 (for legend see opposite page)

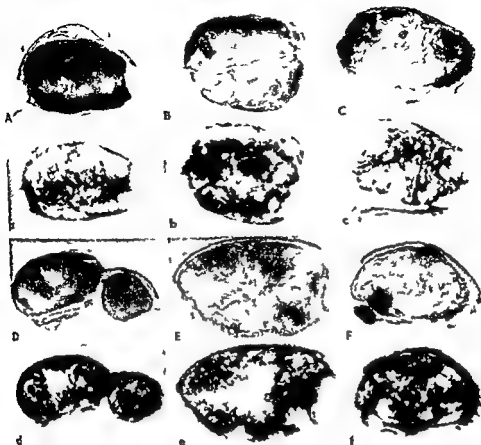


Fig 7 Photomicrographs and radioautographs from kinetic study of uptake after tumor heating at 45°C—30. Magnification $\times 11$.

A—a) B—b) and C—c) Control tumors (RT—30) 1, 2 and 3 days. The light pink staining medullary area expands to occupy more of tumor as time passes. Radioactivity located predominately in this light medullary area and in transition zone immediately surrounding it. Radioactivity becomes more widespread with time as it follows the light pink stained area.

D—d) E—e) and F—f) Treated tumors (45°C—30) 1, 2 and 3 days. Radioactivity widespread throughout tumor on first day after treatment only a small area of medullary tissue is low in radioactivity. At one day after treatment (D—d) the cortex and medulla are not clearly demarcated and whole tumor stains pinkish blue. Cellular structure fairly regular with polygonal tumor cells having moderately large nuclei interspersed in pink fibrinous material. On second and third days after treatment (E—e and F—f) two definite zones appear. Radioactivity is localized mainly in the light pink staining regions characteristic of the medulla and is characteristically absent from the dark blue staining areas that might represent tissue not affected by heat treatment since they usually lie on the side of the tumor which was next to the body.

Table 2

Kinetics of tumor fibrinogen localization after heating at 45 C—30 — 24 hour uptakes starting at various times in relation to treatment

Treatment	Number of rats	%ID/gTN	Therapeutic ratio	Whole body radioactivity retained (%)	Average rat weight gram	Average tumor weight gram
Control R 1 30 injected prior to treatment	8	1.77 ± 0.29	4.2 ± 0.5	51.1 ± 3.9	150 ± 7	5.55 ± 0.53
Control injected following treatment	5	1.99 ± 0.08	4.0 ± 0.2	57.9 ± 1.3	148 ± 4	2.12 ± 0.33
Control injected 24 hours following treatment	5	1.87 ± 0.23	1.7 ± 0.6	15.1 ± 1.8	154 ± 3	2.06 ± 0.53
45 C—30 injected prior to treatment	7	4.34 ± 0.19	12.0 ± 0.8	53.6 ± 2.1	147 ± 7	5.69 ± 1.33
45 C—30 injected following treatment	5	6.94 ± 1.11	12.8 ± 1.7	67.5 ± 3.9	151 ± 3	3.38 ± 0.61
15 C—30 injected 24 hours following treatment	5	1.65 ± 0.29	3.8 ± 0.8	50.1 ± 1.0	145 ± 5	2.21 ± 0.98

area thought to be necrotic and which stained pink in hematoxylin and eosin. In the heated tumors, radioactivity was also found in areas staining pink in hematoxylin and eosin but this area occupied a greater portion of the tumor and usually included the tumor cortex. Microscopic examination of sections of heated tumors and their radioautographs suggests that ^{125}I radioactivity is fixed in a lightly pink stained filamentous network located outside blood capillaries and sinuses. It is difficult, however, to pin point the source of radioactivity at a cellular level with the radioautograph procedure here employed.

^{125}I albumin localization in the heated Walker carcinoma Nine days after Walker tumor transplant, 29 rats were given 5% EACA-KI as drinking water. On the tenth day after transplant each rat was given about 100 μCi of ^{125}I albumin intravenously. As soon thereafter as possible, the tumors of 14 experimental animals were treated in 45° C water for 30 minutes as previously described. Tu

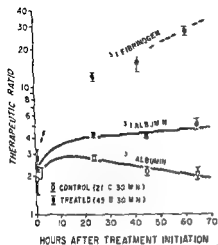


Fig 9 Comparison of ^{131}I fibrinogen and ^{131}I albumin therapeutic ratios versus time after tumor heating

treatment was such that the internal tumor temperature reached approximately 44°C and was maintained at that or greater temperatures for several minutes. It is possible that this temperature range must be reached before bradykinin or some other mediator of increased capillary permeability is released in the heated tumor tissue.

Although treatment for 10 or 20 minutes at 50°C caused the most marked increase in tumor fibrinogen localization encountered in the warm water study, this treatment temperature caused considerable injury to normal tissue and was consequently excluded from further investigation.

In general, control tumors exhibited a necrotic region in their center surrounded by a zone in which the cells were starting to undergo necrosis presumably because of an insufficient blood supply. Around this region the tumor cortex composed of viable tumor cells and stroma was situated. The radioactivity in control tumors was usually located at interfaces between tumor and normal tissue and in the zone between the viable tumor cortex and the necrotic tumor core. It seems reasonable that materials released from dying cells induced an inflammatory reaction in the neighboring regions and thus blood-borne ^{131}I fibrinogen was deposited there. When heat was applied to tumor tissue, the metabolic demands of the viable tissues were increased and their blood supply, already being under considerable stress, was insufficient for the demand and the cells died. In such regions, cellular materials released by autolysis could increase the low-grade inflammatory reaction occurring and induce extravasation and clotting of blood-borne fibrinogen. Thus, an uneven distribution of ^{131}I deposition would be expected and would depend on how close to being overstressed the

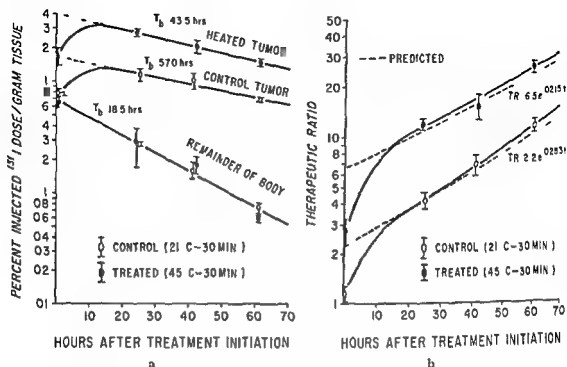


FIG. 8 Specific ^{131}I fibrinogen activity of tumor and carcass versus time after heating in (a) 21°C water (a) and therapeutic ratio versus time after treatment (b)

mors of 15 control animals were treated for the same length of time but with the water bath at 21°C . Immediately after this treatment, two experimental and three control rats were sacrificed and the previously described parameters were determined. At 24, 45 and 65 hours after treatment initiation, four experimental and four control rats were sacrificed and the usual parameters were calculated. The results of this study are plotted in Figs 9 and 10.

Discussion

The stated object of this series has been to investigate a variety of methods by which the localization of radioiodinated fibrinogen in the Walker carcinoma 256 might be enhanced. Results obtained in the course of this investigation suggest that selective heating of a Walker tumor markedly increases the localization of previously injected ^{131}I fibrinogen in the tumor. The tumor had to be exposed to an environment of about 45°C for at least 20 minutes to obtain significantly greater localization of ^{131}I fibrinogen within the tumor.

It is tempting to apply the findings of ROCHA & SILVA (1963) to the problem of tumor fibrinogen localization after heat treatment. No significant increase in tumor fibrinogen localization above the control level was noted until heat

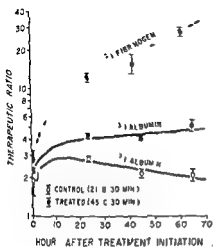


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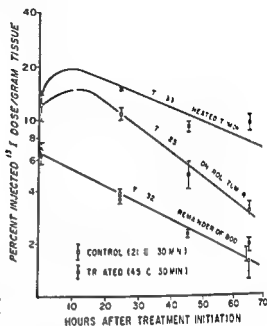


Fig. 10 Specific activity of ^{125}I albumin in tumor and carcass versus time after heating in 45°C water (conditions as in fig. 8)

circulation of particular tumor areas was. Radioautographs of heated tumors evidenced a radioactivity deposition which became more widely distributed as the amount of heat treatment was increased.

A theoretical analysis of tumor fibrinogen localization after treatment in 45°C water for 30 minutes is presented in Fig. 8. On the left of the figure, the logarithms of percentage of injected dose per gram ($\% \text{ ID/g}$) of heated and control tumor tissue and for the carcasses of heated and control rats are plotted against time. These curves are consistent with the hypothesis that ^{125}I fibrinogen is deposited in the tumor primarily during the first 20 hours after injection whether the tumor is heated or not, and that thereafter tumor radioactivity concentration decreases exponentially with time. Because the $\% \text{ ID/g}$ data contain no component of ^{125}I physical decay, the exponential decrease of tumor radioactivity concentration with time indicates a removal of ^{125}I from the tumor at a rate proportional to the amount present.

The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor. The carcass radioactivity concentration is approximately the same initially in the treated and control groups and the carcass biologic half life of ^{125}I fibrinogen is equal in the treated and control groups.

The straight line portions of the $\log \% \text{ ID/g}$ versus time plots can be assigned mathematical descriptions based on the exponential relation $I_t = A_0 e^{-\lambda t}$ where A_0 is tissue radioactivity concentration, $\% \text{ ID/g}$ at any time t . For the tumors, the

relation holds when $t \gg$ greater than about 20 hours A_0 is the hypothetical initial tissue radioactivity concentration, λ is the biological decay constant in hours⁻¹ and is equal to $0.693/T_1$ where T_1 is the biologic half life in hours of ^{131}I fibrinogen in the tissue in question. The time in hours after treatment initiation is represented by t .

When the suitable constants determined in Fig. 6 are substituted into the above relation the following mathematical descriptions for tissue radioactivity versus time are obtained

treated tumor	$A_t = 4.0e^{-0.159t}$	(a)
control tumor	$A_t = 1.5e^{-0.1t}$	(b)
treated carcass	$A_t = 0.62e^{-0.375t}$	(c)
control carcass	$A_t = 0.68e^{-0.375t}$	(d)

The therapeutic ratio as a function of time for control and treated groups can be calculated from relations b, d and a, c, respectively

$$\text{T.R. control} = \frac{A_t (\text{control tumor})}{A_t (\text{control carcass})} = 2.2e^{0.53t}$$

$$\text{T.R. treated} = \frac{A_t (\text{treated tumor})}{A_t (\text{treated carcass})} = 6.5e^{0.15t}$$

These calculated theoretical expressions for the therapeutic ratios are plotted as functions of time on the right side of Fig. 8. The variations of the experimental therapeutic ratios with time are also plotted here as they were in Fig. 6. The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference of biologic half life of ^{131}I fibrinogen in the tumor and carcass.

In order to assess the effect of the warm water treatment on the average total radiation doses to tumor and carcass the concept of effective half life must be included

$$T_{\text{effective}} = \frac{T_b \times T_p}{T_b + T_p}$$

where T_b = biologic half life (just determined), T_p = physical half life (192 hours for ^{131}I)

since $D_p(\infty) \approx \text{constant} \times C_0 \times T_{\text{eff}}$ (adapted from LOEWINGER et coll.)

where $D_p(\infty)$ is the average total radiation dose from ^{131}I β s absorbed by a tissue in which the initial radioactivity concentration is C_0 and the effective half life of ^{131}I is T_{eff} . The ratio of total tumor dose to total carcass dose is thus

$$\frac{D_{\beta}(\infty) \text{ tumor}}{D_{\beta}(\infty) \text{ carcass}} = \frac{C_0 \times T_{\text{eff}} \text{ (for tumor)}}{C_0 \times T_{\text{eff}} \text{ (for carcass)}} = T R_0 \times \frac{T_{\text{eff(tumor)}}}{T_{\text{eff(carcass)}}}$$

$$\frac{\text{average } \beta \text{ dose to tumor}}{\text{average } \beta \text{ dose to carcass}} = \frac{6.5 \times 2.1}{2.2 \times 2.6} = \frac{13.7}{5.7} \text{ for treated group}$$

This theoretical calculation indicates that the warm water treatment improved the ^{131}I fibrinogen radiation therapy of the Walker tumor nearly 250 per cent.

Preliminary experiments by BAILE et al. (1964) have indicated that heating rat transplanted Walker tumors at 41.5°C for five hours markedly increases tumor localization of ^{131}I labeled antifibrin antibody.

Several conclusions can be drawn from ^{131}I albumin results. The therapeutic ratio for fibrinogen injected tumors (Fig. 9) is nearly six times greater three days after treatment than that for tumors given ^{131}I albumin and identical heat treatment. Data presented in Fig. 10 suggest two reasons for this difference in therapeutic ratios. Although the biologic half lives of ^{131}I albumin and ^{131}I fibrinogen are approximately the same in the heated Walker tumor, the maximum tumor radioactivity concentration is nearly two times greater after ^{131}I fibrinogen administration. In addition, the biologic half life of radioalbumin in the non tumor portion of the rat's body is almost twice as long as that of radiofibrinogen. These factors combine to produce considerably less increase of therapeutic ratio with time in the ^{131}I albumin injected rats.

This study seems to indicate that proteins such as fibrinogen and antifibrin antibody whose tumor localization is associated with the clotting mechanism, are unusual in their susceptibility to be retained in the heated Walker tumor without marked concurrent retention in the non tumor portions of the host.

Acknowledgement

This work is based on a thesis submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy in Radiation Biology at the University of Rochester and was supported in part by the Atomic Energy Commission and in part by a National Institute of Health training grant in Biophysics. The deepest appreciation is expressed to William F. Bale, the thesis advisor, for his suggestions and guidance. The author further wishes to thank John Harris for supplying the rat albumin used in this study. The publication has been assigned Report No. UR 49 1265.

SUMMARY

Heating of the Walker carcinoma 256 by immersion in a water bath at 45°C for 30 minutes led to a substantial increase in the amount of intravenously injected ^{131}I fibrinogen which localized in the tumor. Such heat treatment increased the tumor radiation therapy dose from ^{131}I almost 250 per cent. Heated muscle in the neighborhood of tumor did not localize ^{131}I fibrinogen. Radioactivity deposition coincided with histologically demonstrable areas of tumor damage. ^{131}I albumin was not effectively cleared from non tumor tissues.

ZUSAMMENFASSUNG

Eine Erwärmung des Walker Carcinoms 256 durch Eintauchen in ein Wasserbad von 45 C 30 Minuten lang führt zu einem kräftigen Anstieg der Menge intravenös injizierten ^{125}I Fibrinogens die sich im Tumor ansammelt. Eine derartige Wärmebehandlung steigert die therapeutische Strahlendosis des Tumors durch ^{125}I um beinahe 250 %. Die erwärmte Muskulatur der Umgebung des Tumors nimmt nicht ^{125}I Fibrinogen auf. Die Ablagerung der Radioaktivität stimmt mit der histologisch nachweisbaren Ausbreitung der Tumorschädigung überein. ^{125}I Albumin wurde nicht effektiv von den nicht Tumor Geweben befreit.

RESUMÉ

Élévation de température dans un carcinome 256 de Walker par immersion dans un bain d'eau à 45 C pendant 30 minutes augmente de façon importante la quantité de fibrinogène marqué par ^{125}I injecté par voie intraveineuse qui se localise dans la tumeur. Ce traitement thermique augmente presque de 250 pour cent la dose d'irradiation fournie par ^{125}I le muscle chauffé dans le voisinage de la tumeur ne fixant pas le fibrinogène ^{125}I . La fixation de la radio-actiue coïncide avec des dommages à la tumeur mis en évidence par l'histologie. L'albumine marquée par ^{125}I n'est pas déplacée hors des tissus non tumoraux par ce traitement thermique.

REFERENCES

- BALE W F, SPAR I L and GOODLAND R L. Experimental radiation therapy of tumors with ^{125}I carrying antibodies to fibrin. *Cancer Res* 20 (1960) 1488
- — — Research directed toward the use of ^{125}I labeled fibrinogen and antibody to fibrin in the localization and treatment of tumors. Univ. of Rochester Atomic Energy Project Report (1967) UR 617
- HELMKAMP R W, DAVIS T P et coll. High specific activity labeling of protein with ^{125}I by the iodine monochloride method. *Proc Soc exp Biol (N.Y.)* 122 (1966) 407
- IZTO M J, GOODSON L L et coll. Effect of heat on localization of ^{125}I antibody to rat fibrin in Walker 256 carcinosarcomas. *Fed Proc* 23 (1964) 288
- COLLIN E J, STRONG L E, HUGHES JR W L et coll. Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J Amer chem Soc* 68 (1946) 459
- CRILE JR G. The effects of heat and radiation of cancers implanted in the feet of mice. *Cancer Res* 23 (1963) 372
- DAY E D, PLAVISER J A and PRESSMAN H. Localization of radioiodinated rat fibrinogen in transplanted rat tumors. *J nat Cancer Inst* 23 (1959) 799
- DEWEY W C, BALE W F, ROSE R G and MARRACK D. Localization of antifibrin antibodies in human tumors. *Acta Un int Cancr* 19 (1963) 185
- DI CHIRO G, SPAR I L, BALE W F et coll. RIAF—radio-iodinated anti fibrinogen encephalography. *Acta radiol Diagnosis* 1 (1963) 967
- FARLE W B. A study of the Walker rat mammary carcinoma 256 in vivo and vitro. *Amer J Cancer* 24 (1935) 566
- FISHER E R and FISHER B. Electron microscopic, histological and histochemical features of the Walker carcinoma. *Cancer Res* 21 (1961) 577

- HEIMKAMP R. W., GOODLAND R. L., BALL W. F. et coll. High specific iodination of gamma globulin with iodine 131 monochloride. *Cancer Res.* 20 (1960), 1495.
- HIRAMOTO R., BERNICKY J., JURADOWSKI J. and PRESSMAN D. Fibrin in human tumors. *Cancer Res.* 20 (1960) 592.
- LOVINGER R., HOIT J. G. and HINE G. J. Internally administered radioisotopes. In *Radiation Dosimetry*, p. 867. Edited by C. J. Hine and G. L. Brownell. Academic Press, New York, 1956.
- MCCARDLE R. J., HARRIS P. V., SPAR I. I. et coll. Studies with iodine 131 labeled antibody to human fibrinogen for diagnosis and therapy of tumors. *J. nucl. Med.* 7 (1966) 837.
- MICHAEL S. I. The isolation of albumin from blood serum and plasma by means of organic solvents. *Biochem. J.* 82 (1962) 212.
- MUTSCHLER I. F. The effect of orally administered epsilon aminocaproic acid on the deposition and/or retention of radioiodinated fibrinogen and antibodies to fibrinogen in subcutaneous rat plasma clots and turpentine induced abscesses of the rat. Thesis, University of Rochester, New York, 1963.
- Anti fibrinolytic effect of epsilon aminocaproic acid as measured by *in vivo* clot lysis. *Proc. Soc. exp. Biol. (N.Y.)* 115 (1964) 1019.
- ROCHA E SILVA M. The physiological significance of bradykinin. *Ann. N.Y. Acad. Sci.* 104 (1963) 190.
- SCHALLER J. R. Evidence for the participation of the coagulation mechanism in the localization of ¹³¹I fibrinogen in a transplantable rat tumor. Thesis, University of Rochester, New York, 1962.
- Interference in localization of ¹³¹I fibrinogen in rat tumors by anticoagulants. *Amer. J. Physiol.* 206 (1964) 573.
- SOARDI F. e MAICHIANO A. L'emocoagulazione in ratti portatori di tumore di Walker. (In Italian). *Haemat. (Pavia)* 44 (1959) 137.
- VARON M. I. and SPAR I. I. The increase in uptake of ¹³¹I labeled fibrinogen in tumor tissue by modification of the coagulation mechanism of the host. *Proc. Amer. Ass. Cancer Res.* 6 (1965) 66.
- WARREN W. L. Effect of artificial fever upon hopeless tumor cases. *Amer. J. Roentgenol.* 33 (1935) 75.

CELLULAR AUTOIMMUNE REACTIONS FOLLOWING RADIOIODINE TREATMENT FOR HYPERTHYROIDISM

by

NINA LINNORH J WASSERMAN and T PACKALEN

Radioiodine therapy is known to be followed by an increase in the circulating thyroid antibodies (BUCHANAN et coll 1962 IRVINE 1964 O GORMAN et coll 1964 LINNORH et coll 1965). In hyperthyroidism there may be an increase in antibodies to thyroid cytoplasmic antigen, though not to thyroglobulin (LINNORH et coll 1965 JOHANSSON et coll 1968) and in euthyroidism an increase in antibodies to both thyroid cytoplasmic antigen and thyroglobulin (LINNORH et coll 1966). This increase in the circulating antibodies is temporary and lasts about a year after ^{131}I therapy (LINNORH et coll 1965). A significantly higher frequency of positive serologic reactions has occurred in patients developing hypothyroidism within one year of ^{131}I treatment for hyperthyroidism (LINNORH et coll 1965).

It is generally believed that the specific tissue damage occurring in the autoimmune states is mediated by cellular reactivity. It would therefore be of interest to know whether patients treated with radioiodine develop cellular reactivity to thyroid antigens, as happens in autoimmune states. This knowledge

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might provide information on whether the autoimmune reaction induced by the local irradiation is of significance for the effect of radiation. The response of lymphocytes from such patients to thyroglobulin has been studied *in vitro*.

Thyroglobulin has been preferred to the microsomal antigens in order to keep allotypic differences to a minimum. The stimulation of lymphocytes *in vitro* in the presence of antigen seems not to be closely dependent on circulating antibodies although in some way related to the state of delayed hypersensitivity (MILLS 1966, ORENHIM *et al.* 1967). No definite proof has been presented, however, that such stimulation is a manifestation of delayed hypersensitivity.

During recent years several methods of examining lymphocytic reactions to antigens *in vitro* have been developed. One method relies on the fact that an increase in the incorporation of isotope labelled thymidine by the cells indicates an increase in DNA synthesis in the presence of antigen (LING 1968). This method has been used in the experiments now reported. The stimulation of DNA synthesis by PPD tuberculin was tested on lymphocytes from subjects with positive skin reactivity to tuberculin to verify that the method was adequate when an active stimulant was used. Lymphocytes and the thyroid glands of guinea pigs immunized with human thyroglobulin in complete adjuvant were studied in addition to lymphocytes from patients treated with radioiodine.

Material and Methods

Human lymphocyte donors. The material consisted of 25 subjects divided into three groups comprising 11 healthy controls, 8 patients who had previously received radioiodine therapy and 6 patients with subacute thyroiditis.

The patients given radioiodine were all selected as having circulating antibodies to thyroglobulin or thyroid cytoplasmic antibodies at the time of examination. Their passive haemagglutination titres ranged from 1/80 to 1/12 500, four of them also had antibodies to cytoplasmic antigen as assessed by the fluorescent antibody (FA) technique. Seven of the patients were women and one was a man, their ages ranged from 49 to 69 years. They were examined 1–4 years after radioiodine treatment for hyperthyroidism, four of them having diffuse and four nodular goitres. The total dose of radioiodine ranged from 3.5 to 33 mCi ^{131}I .

All the six patients with clinical signs of subacute thyroiditis had antibodies (FA) to microsome antigen, and four of them had haemagglutinating antibodies to thyroglobulin in titres ranging from 1/400 to 1/4 000 000. The five women and one man were from 29 to 43 years of age.

Eleven subjects, nine women and two men, aged between 24 and 48 years

were taken as controls none had demonstrable humoral antibodies to thyroid antigens

Experimental animals Nine male guinea pigs weighing about 400 g were immunized by injecting 2 mg of human thyroglobulin in complete Freund's adjuvant as described for the immunization of this species with guinea pig thyroglobulin (WASSERMAN & PACRALEN 1965). After 3 weeks the skin hyper-sensitivity to thyroglobulin was examined and the animals killed, the thyroid glands then being examined for histologic changes and the lymphocytes for stimulation of the DNA synthesis in the presence of thyroglobulin. Untreated male guinea pigs were used as controls.

Methods Thyroglobulin was prepared from human thyroid tissue removed during surgery by the method described by LINDER & WEISULL (1960). Several batches each from one single gland, were used. PPD tuberculin (purified protein derivative) (kindly supplied by Parke Davis & Co Detroit Michigan) without preservatives was employed. One hundred milligrammes of phytohaemagglutinin were dissolved in 5 ml Hank Tris buffer and frozen in small portions. About 50 to 100 ml of blood were withdrawn from a cubital vein of the patients and controls and defibrinated by cautious agitation with glass beads. The lymphocytes were isolated from the defibrinated blood by the method of COULSON & CHALMERS (1964). The cells were washed twice in Hank Tris buffer, counted in a Barker chamber and suspended in Eagle's medium supplemented with 10% heat inactivated autologous serum or fetal calf serum. One million lymphocytes were pipetted into conical tissue culture tubes. The antigens in appropriate concentrations were added to the cells in a volume of 0.5 ml or 250 to 500 μ g of PHA M in a volume of 0.5 ml.

Tubes without antigens served as controls. The total volume of the incubation mixtures was 1.0 ml. The tubes were loosely closed with screw caps and incubated with a continuous flow of a mixture of 5% carbon dioxide in air. After 72 hours 0.4 μ Ci of 14 C thymidine (specific activity 35.7 mCi/mM or 54.4 mCi/mM) was added to all tubes. After a further 24 hours the tubes were cooled in ice water and washed 4 times in cooled buffer. One drop of saturated 14 C thymidine was added to the first two washings. The washed cells were dissolved in 1.0 ml of concentrated formic acid and the liquid was transferred to planchets. After drying, the radioactivity was determined in a gasflow counter equipped with a Geiger Muller tube. The uptake of 14 C thymidine in each sample was expressed in counts per minute and the means of the values for duplicate tubes were taken. The results were expressed as the ratio between the counts per minute obtained with and without antigen. This ratio was called the lymphocyte stimulation index (LSI).

Spleen cells were used in the guinea pig experiments in place of blood lymphocytes. The spleen was removed aseptically and cut into small pieces.

Suspensions of spleen cells were prepared by mincing the tissue in culture medium. In every other respect the method was the same as that used in the experiments with human blood lymphocytes. The stimulation tests were occasionally performed twice, with either autologous serum or fetal calf serum added to the culture medium. The results were the same for the two media.

When thyroglobulin was used as the antigen, 10, 100, 1 000 or 5 000 mcg were added to each tube in eight subjects, three of these concentrations of antigen were used in an additional thirteen subjects. In fourteen of these subjects the highest stimulation of the DNA synthesis was observed in the tubes to which 1 000 mcg thyroglobulin had been added, in three in the tubes to which 100 mcg and in four subjects in the tubes to which 10 mcg had been added. The stimulation was never better with 5 000 than with 1 000 mcg, only one preparation with 1 000 mcg of thyroglobulin per tube was therefore tested. With PPD as antigen 5 mcg were added per tube.

Tubes with PHA M were included as positive controls in each experiment. The phytohaemagglutinin always strongly stimulated DNA synthesis as measured by the uptake of ^{14}C thymidine.

Humoral antibodies to thyroglobulin and thyroid cytoplasmic antigens were tested at the National Bacteriologic Laboratory (JONSSON *et al.* 1968).

The time after treatment was counted from the last radioiodine dose in patients given more than one dose of radioiodine.

Results

The mean of the lymphocyte stimulation index in the healthy controls was 1.04 ± 0.11 , in the patients receiving radioiodine therapy 1.45 ± 0.25 and in the patients with subacute thyroiditis it was 1.50 ± 0.23 , the differences between these groups were not statistically significant. However, in the presence of thyroglobulin the lymphocyte stimulation index ranged in healthy donors from 0.62 to 1.57 (Table 1), and in the radioiodine treated patients from 0.70 to 3.00. Three of the eight patients had an index higher than that for any of the eleven healthy controls (Ht3, Ht4, Ht5, Table 2).

The index ranged from 0.90 to 2.30 in subacute thyroiditis and in two of the six patients the value was higher than for any of the eleven healthy controls (T4 and T6, Table 3).

No correlation between the values of the lymphocyte stimulation index in the presence of thyroglobulin and of humoral antibodies to thyroglobulin, as tested by passive haemagglutination of tanned cells could be found.

Table 1

Lymphocyte stimulation index (LSI) in the healthy controls — Mean of LSI = 1.04 ± 0.11 (standard error of the mean)

Subjects	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
LSI	1.57	1.37	0.39	1.09	0.97	1.40	0.62	0.86	0.71	1.41	1.05

Table 2

Lymphocyte stimulation index (LSI) in the patients treated with ^{131}I for hyperthyroidism — Mean of LSI = 1.43 ± 0.25 (standard error of the mean)

Subjects	Ht1	Ht2	Ht3	Ht4	Ht5	Ht6	Ht7	Ht8
LSI	1.14	1.09	1.63	1.69	3.00	1.30	0.70	0.90
Years after radiotherapy	1	3	1	3	4	3	2	3
Total ^{131}I dose mCi	33	27	12	3.5	27	6	5	3.2

Table 3

Lymphocyte stimulation index (LSI) in Hashimoto thyroiditis — Mean of LSI = 1.50 ± 0.23 (standard error of the mean)

Subjects	T1	T2	T3	T4	T5	T6
LSI	1.40	0.95	1.43	2.04	0.90	2.30

The addition of PPD tuberculin stimulated DNA synthesis in both lymphocyte donors with a lymphocyte stimulation index of 4.9 and 7.0; these controls gave positive skin tests to tuberculin in a dilution of 1:1,000.

When the human thyroglobulin used in the above stimulation study was injected with complete Freund's adjuvant into the 9 guinea pigs, a delayed type skin sensitivity to thyroglobulin developed. Stimulation of DNA synthesis in the presence of thyroglobulin was demonstrated in lymphocytes from most of the animals. Morphologic signs of thyroiditis were observed in the four submitted to histologic examination.

Discussion

It is evident from the stimulation of DNA synthesis by PPD tuberculin that the experimental system employed in this study was effective when the stimulant was active.

Immunization of the guinea pigs with human thyroglobulin in complete adjuvant produced the usual sequence of events (McMASTER et coll 1961, WASSERMAN & PACKALEN 1965), of particular interest was the development of thyroiditis. Delayed hypersensitivity is a specific immunologic manifestation induced by many heterologous antigens incorporated in complete adjuvant. The induction of thyroiditis points to an organ specific activity of the thyroglobulin preparation used.

The results of the experiment with lymphocytes from patients with thyroiditis are in agreement with those of other workers. Only occasional positive results have been reported (LACETTE & PEARMAIN 1965, FORBES 1965). DE GROOT & JAKSINA (1969) in Hashimoto thyroiditis observed no stimulation of the thymidine incorporation in lymphocytes neither by thyroid supernatant fraction nor by thyroid microsomal fraction.

The main object of the present experiments was to ascertain whether lymphocytes from patients treated with radioiodine and displaying circulating antibodies to thyroid antigens were sensitized to thyroglobulin. The groups composing the material are small and the results preliminary. However, three of the eight patients receiving radioiodine therapy for hyperthyroidism had a higher lymphocyte stimulation index than any of the eleven healthy controls, and one recorded a value higher than the mean for the control group ± 5 times the standard error. This increase in the stimulation of blood lymphocytes to thyroglobulin in some of the radioiodine treated patients may either be due to the effect of this local radiotherapy to the thyroid gland or may have been present before the radioiodine had been given. A considerable proportion of untreated patients with hyperthyroidism have demonstrable antibodies to thyroglobulin and to cytoplasmic antigens, no information is, however, available on the lymphocyte stimulation index in untreated hyperthyroidism (DE GROOT & JAKSINA 1969).

It has previously been reported (EINIÖRN et coll 1965, JONSSON et coll 1968) that the increase in circulating thyroid antibodies after radioiodine therapy for hyperthyroidism is confined to antibodies to thyroid cytoplasmic antigens and is temporary, lasting about a year. There is no information as to whether the cellular reactivity in this respect follows the serologic reactivity. All the patients in this series had been treated with radioiodine more than a year previously.

Acknowledgements

The authors take this opportunity of thanking Prof. Fagrieus as well as Drs. Jonsson, Ljunggren, Almquist and Wijnbladth for their cooperation in the work. The PPD tuberculin was supplied by Parke Davis, Detroit, Michigan and the phytohemagglutinin by PHARM Linc Laboratories, Detroit, Michigan.

SUMMARY

A material of 25 subjects including eight patients who had previously received radioiodine therapy was examined to determine whether cellular reactivity to thyroid antigens developed. The results which are preliminary are discussed in detail.

ZUSAMMENFASSUNG

An einem Material von 25 Individuen einschliesslich acht Patienten die vorher mit radioaktivem Jod behandelt worden waren wurden Untersuchungen angestellt um zu ermitteln ob Zeichen einer zellularen Reaktion gegen Schilddrüsenantigene gefunden werden konnten. Die Resultate die preliminar sind werden diskutiert.

RÉSUMÉ

Les auteurs ont cherché s'il apparaît une réactivité cellulaire aux antigènes thyroïdiens chez 25 sujets dont huit malades avaient été traités par l'iode radio-actif pour hyperthyroïdie. Ils examinent en détail les résultats qui sont préliminaires.

REFERENCES

- BICHANAY W W, KOUTRAS D A, CROOKS J et coll. The clinical significance of the complement fixation test in thyrotoxicosis. *J. Endocr.* 24 (1962) 115.
- COLLISON A S and CHALMERS P G. Separation of viable lymphocytes from human blood. *Lancet* 1964 *i* p 468.
- DE GROOT L J and JAKSHAS S. Observations on the role of circulating lymphocytes in thyroid auto-immunity. *J. clin. Endocr.* 29 (1963) 207.
- ERIKSSON J, FAGRAELS A and JOHANSSON J. Thyroid antibodies after ^{131}I treatment for hyperthyroidism. *J. clin. Endocr.* 25 (1958) 1218.
- — — Thyroid antibodies in euthyroid subjects after iodine ^{131}I therapy. *Radiat. Res.* 28 (1965) 296.
- ERIKSSON J, JOHANSSON J and FAGRAELS A. Immunologic reactions after irradiation of the uterus. To be published in *Radiat. Res.*
- FORBES J J. Specific and non specific stimulation of antibodies synthesis by human leucocytes in vitro. *Lancet* 1965 *i* p 198.
- IRVINE W J. Thyroid auto immunity as a disorder of immunological tolerance. *Quart. J. exp. Physiol.* 49 (1964) 374.
- JOHANSSON J, ERIKSSON J, FAGRAELS A and ERIKSSON J. Organ antibodies after local irradiation. *Radiology* 90 (1968) 536.
- LIVE N R. Lymphocyte stimulation. North Holland Publishing Co. Amsterdam 1968.
- LYFFLE R R and FEARMAN G E. Lymphocyte cultures in rheumatoid arthritis and thyroid diseases. *N. Z. med. J.* 64 (1965) 81.
- McMASTER I P D, LERNER E M and ELLIS E D. The relationship of delayed hypersensitivity and circulating antibody to experimental allergic thyroiditis in inbred guinea pigs. *J. exp. Med.* 113 (1961) 611.

Immunization of the guinea pigs with human thyroglobulin in complete adjuvant produced the usual sequence of events (McMASTER *et coll* 1961, WASSERMAN & PACKALEN 1965), of particular interest was the development of thyroiditis. Delayed hypersensitivity is a specific immunologic manifestation induced by many heterologous antigens incorporated in complete adjuvant. The induction of thyroiditis points to an organ specific activity of the thyroglobulin preparation used.

The results of the experiment with lymphocytes from patients with thyroiditis are in agreement with those of other workers. Only occasional positive results have been reported (LYCETTE & PEARMAIN 1965, FORBES 1965). DE GROOT & JAKSINA (1969) in Hashimoto thyroiditis observed no stimulation of the thymidine incorporation in lymphocytes neither by thyroid supernatant fraction nor by thyroid microsomal fraction.

The main object of the present experiments was to ascertain whether lymphocytes from patients treated with radioiodine and displaying circulating antibodies to thyroid antigens were sensitized to thyroglobulin. The groups composing the material are small and the results preliminary. However, three of the eight patients receiving radioiodine therapy for hyperthyroidism had a higher lymphocyte stimulation index than any of the eleven healthy controls, and one recorded a value higher than the mean for the control group ± 5 times the standard error. This increase in the stimulation of blood lymphocytes to thyroglobulin in some of the radioiodine treated patients may either be due to the effect of this local radiotherapy to the thyroid gland or may have been present before the radioiodine had been given. A considerable proportion of untreated patients with hyperthyroidism have demonstrable antibodies to thyroglobulin and to cytoplasmic antigens, no information is, however, available on the lymphocyte stimulation index in untreated hyperthyroidism (DE GROOT & JAKSINA 1969).

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APPLICATORS FOR REMOTE AFTERLOADING TECHNIQUE FOR OPTIMUM PELVIC DOSE DISTRIBUTION IN CARCINOMA OF THE UTERINE CERVIX

by

INGEMAR JOELSSON and ANDERS BACKSTROM

Attempts at reaching optimum dose distribution in the pelvis from intra-cavitary radiation for carcinoma of the uterine cervix have interested the clinician for many years and a wealth of literature is available on the subject. Traditionally an improved dose distribution is aimed at by an increase in the source surface distance, an elongation of the intra-uterine source and by using multiple radiation sources preferably spread out in the superior part of the vagina. Lack of space often imposes limitations and difficulties are encountered regarding the maintenance of the position of the sources during the treatment time both in their interrelationship and to the anatomic configurations of the patient.

The design by NEARY (1947) of a combined intra-uterine and intravaginal high activity irradiator employing specific screens in the direction of the bladder and the rectum represents one attempt to achieve improvement in the dose at

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- MIESCHER P, GORSTEIN F, BENACERRAF H and GELL P G H. Studies on the pathogenesis of experimental immune thyroiditis. *Proc Soc exp Biol (N Y)* 107 (1961) 12.
- MILLS J A. The immunological significance of antigen — induced lymphocyte transformation in vitro. *J Immunol* 97 (1966) 239.
- OGORMAN P, STAFFURTH J S and BALLENTYNE M R. Antibody response to thyroid irradiation. *J clin Endocr* 24 (1964) 1072.
- OPPENHEIM J J, WOLSTENCROFT R A and GELL P G H. Delayed hypersensitivity in the guinea pig to a protein — haptan conjugate and its relationship to in vitro transformation of lymph node spleen thymus and peripheral blood lymphocytes. *Immunology* 12 (1967) 89.
- WASSERMAN J and PACKALÉN T. Immune responses to thyroglobulin in experimental allergic thyroiditis. *Immunology* 9 (1965) 1.
- WEINIL C and LINDER L. Preparation and properties of human thyroid globulin. *Acta chem scand* 14 (1960) 2063.

Table 1

Physical characteristics of various materials for the attenuation of radiation from ^{137}Cs

	Z number	Atomic weight	Density	First HVL in mm (^{137}Cs)
Uranium	92	238	18.8	2.9*
Tungsten	74	184	19.3-19.7	4.0 and **
Lead	82	207	11.0	5.5
Platinum	78	195	21.4	3.2 **
Gold (pure)	79	197	19.3	3.6* *
Gold (18 carat)	71***		16.7	4.2* *

According to THORAPES 1965

* Tungsten alloy density 17.7

** Calculated from values in GRODSTEIN 1967

*** Calculated in accordance with MAYNARD 1937

been stepwise increased from the previously used content of 120 to 140 mg radium to three times this amount. The vaginal applicator was loaded with a cesium source train of 600 mCi activity. Distribution of the sources at lateral parts of the ring together with tungsten absorbers at selected locations resulted in a dose reduction in the direction of the urinary bladder and the rectum. A radium source train of variable activity (100 to 250 mg Ra) was used for the intra uterine sound; this applicator combination will be referred to as system No. 1. The recently constructed vaginal applicator model utilizing additional shielding material was employed together with an intra uterine steel sound both tubes being equipped with cesium source trains. The activity of the sources in each applicator was 600 mCi. This applicator combination will be called system No. 2.

In the design of the vaginal applicator of the No. 2 system various high Z materials were considered (Table 1). Uranium was disregarded because of its high reactivity. Lead had a too high HVL figure and platinum was too costly. Tungsten manufactured in sintered form and used as an alloy with copper and nickel was unsuitable because of difficulties encountered in machining it. Eighteen carat gold which has a high resistance to corrosion and is easy to machine was therefore chosen.

The vaginal applicator of the No. 2 system was intended to be so designed as to reduce the doses in the ventral and dorsal directions thereby shielding the bladder base and the anterior wall of the rectum. It was decided not to change the arrangement of sources and absorbers from the previous design by WALSTAM. It had been found however in a study in 4 patients with the

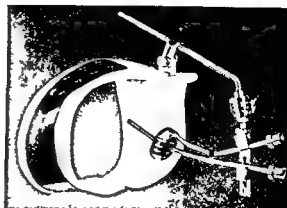


Fig 1 The intracavitary applicators of the remote afterloading unit are held in place by a fibreglas corset

the pelvic wall. More recently proposals have suggested the incorporation of specific shields of high Z metal in the applicators of the current Stockholm method, in combination with the use of medium or low energy gamma radiation sources (KOTTMEIER & WALSTAM 1962). The introduction of the remote afterloading technique opened further prospects. WALSTAM (1965) utilized lateral placement of ^{137}Cs sources surrounded by tungsten absorbers in an early version of an afterloaded vaginal applicator in order to extend the dose distribution laterally. The vaginal applicator was used, however, together with an intra uterine steel sound loaded with radium, it was anticipated that the resulting dose distribution in the pelvis would not differ considerably from that of the current Stockholm technique. Development of an applicator with increased shielding particularly of the bladder and rectum, has therefore continued. One of these applicator models has been introduced into the routine clinical work after determination of the dose distribution in a phantom. The relationship between the doses at the pelvic wall and the dose at the posterior part of the bladder and the anterior wall of the rectum has been established in a series of patients.

Intracavitary applicators. With the Stockholm afterloading technique the intracavitary applicators are attached to the patient by means of a special corset (Fig 1). This arrangement decreases the risk of tissue damage due to pressure even if the applicator is heavy. In addition, the gamma radiation from ^{137}Cs is of fairly low energy, i.e. 0.66 MeV, which reduces the necessary thickness of the material used for shielding purposes. The bar and ball joint system also allows the final adjustment of applicator site to be made after the patient is released from the lithotomy position.

Two separate sets of intracavitary applicators were used, one of these consisted of the ring shaped vaginal applicator described by WALSTAM (1965) together with an intra uterine stainless steel sound. The source activity for this set has

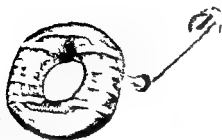


Fig 2 The vaginal applicator of the remote afterloading unit. The gold screens and flanges are covered with araldite.

applicators. The accuracy of this determination was $\pm 3\%$ (standard deviation as percentage of the mean).

It was observed that in a section through the ring the 25 rad/h isodose rate was 78 mm lateral from the center but only 35 mm ventral and dorsal from the center (Fig 3 upper diagram). Ten millimeters ventral or dorsal from the outer surface of the ring the dose rate was less than 25 rad/h while at the same distance lateral from the surface of the ring the dose rate was 125 rad/h. The factor of dose reduction in the ventral and dorsal directions was between 5 and 11.

The 25 rad/h isodose curve was 35 mm ventrally or dorsally to the center of the applicator and 64 mm cranially or caudally to the center in a section perpendicular to the ring (through the shaft of the vaginal applicator). Ten millimeters ventrally from the outer surface the isodose rate was still less than 25 rad/h while 10 mm cranially to the outer surface of the ring the isodose rate was about 150 rad/h. In this plane the factor of dose reduction in the direction of the bladder and the rectum was approximately 6 (see Fig 3 lower diagram).

In clinical practice the vaginal applicator is used in combination with the uterine stainless steel sound which has an outer diameter of 8 mm and a wall thickness of 1 mm. The intra uterine activity (600 mCi ^{137}Cs) is distributed over a length of 58 mm. Six cesium sources each measuring 8 mm \times 3 mm separated into three sections by two spacers each measuring 5 mm \times 3 mm. Uterine steel sounds are available in lengths between 7 and 9 cm measured from the 35° bend (always placed in the center of the vaginal sound) to the distal end. The dose distribution around the combined vaginal and intra uterine applicators was measured in the section perpendicular to the ring through the center of the uterine sound. The dose rates along the bisectrix of the anterior angle between the vaginal ring and the uterine sound were 600, 400 and about 200 rad/h at 10, 20 and 40 mm respectively, with corresponding figures posteriorly of 700, 450 and 200 rad/h (Fig 4 upper diagram). The 50 rad/h isodose rate curve was

Table 2

Doses in rad (mean values and limits of observations in 4 patients) in three parts of 19 mm length and in one part of 6 mm length (under the inguinal ligament) of the right iliac vein during intracavitary radiation treatment with the remote afterloading technique applicator system No 1

	Parts of iliac vein			Under inguinal ligament
	Cranial	Middle	Caudal	
Limits	250—370	336—430	213—410	230—302
Mean	314	400	357	277

intracavitary applicator combination No 1 that the radiation doses in the right common and external iliac veins, determined by thermoluminescence dosimetry, reached a maximum of 400 rad during one course of treatment (Table 2). The mean dose during the same treatment on the posterior wall of the bladder was 2 500 rad and on the anterior wall of the rectum 2 000 rad. The relationship is the same as for the current Stockholm technique. Additional gold shielding was therefore applied to the anterior and posterior parts of the ring of the vaginal applicator of the No 2 system. The gold shielding was shaped into the form of a part collar around the stainless steel sound to produce a sufficiently wide zone of protection. Care was taken to adjust the gold shield as close to the radiation sources as possible in order to increase the spread angle it occupied. Discs of gold were also attached to the periphery of the steel sound between each radiation source. The sizes of the discs were chosen to allow only narrow beams of radiation to reach the surrounding regions. Thus in the lateral direction close to the applicator, a reduced surface dose was obtained since small tissue volumes received radiation from only one radiation source. At greater distance from the ring arbitrarily chosen tissue volumes were found to receive irradiation from all three radiation sources.

The vaginal applicator with the gold shields was covered with waldite. The total diameter of the applicator was 54 mm and the outer diameter of the stainless steel tube was 8 mm (Fig. 2).

The isodose curves around the vaginal applicator were determined by means of an automatic isodose recorder (LARSSON *et al.* 1963) with some modifications. The instrument was equipped with an anthracene crystal placed inside an aluminium tube connected to a photomultiplier. The dependence of the dose response on radiation quality for this crystal was found to be within $\pm 5\%$ in the actual photon energy range. The dose rates were measured with thermoluminescence detectors placed in a perspex fixture firmly attached to the ap-

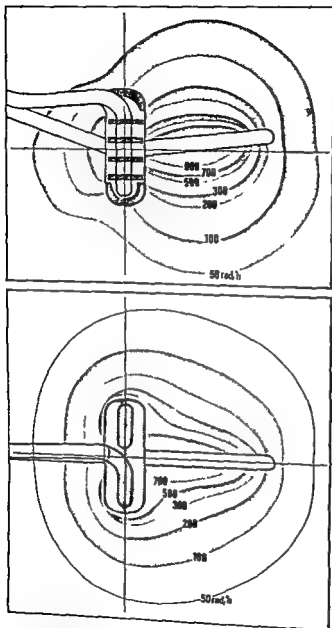


Fig 4 Dose distribution in the sagittal section through the center of the intra uterine sound (upper diagram) and in a frontal oblique plane through the intra uterine applicator (lower diagram) of the combined applicator of the No 2 system.

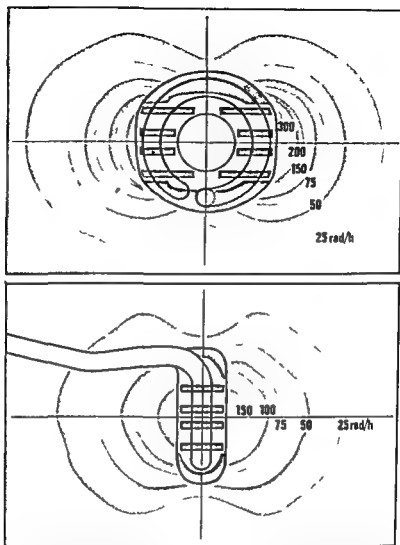


Fig 3 Dose distribution in the plane of the ring of the vaginal applicator (upper diagram) and in a plane perpendicular to the ring of the vaginal applicator (lower diagram) of the No 2 system

45 mm lateral to the outer surface of the vaginal applicator in the section through the intra uterine steel sound which forms an oblique angle with the ring, at a level 20 mm cranial to the external os 700, 310, 200 and 100 rad/h dose rates were recorded 10, 20, 30 and 50 mm, respectively lateral to the center of the intra uterine sound (Fig 4 lower diagram)

Methods Eight patients with I b or II a lesions were subjected to lymphography, phlebography and bilateral placement of thermoluminescence radiation detectors in accordance with techniques described in detail elsewhere (JOELSSON

occurred between the dose rates at the anterior wall of the rectum measured in lithotomy and supine positions (Table 3). The range of differences was large with a minimum of -30% and a maximum of $+94\%$ with a mean of $+18\%$. Six of the 18 patients exhibited differences of over $+20\%$ and for 4 of them the difference amounted to more than $+40\%$. The mean difference of $+18\%$ cannot be explained by inaccuracies in measurements but suggests a true difference between dose rates in the two positions. No such differences were observed in dose rates on the posterior wall of the urinary bladder. For the detailed description of the procedure of dose rate measurement the reader is referred to a previous paper by JOELSSON & BACKSTROM (1969).

Results

The mean values of the doses over one course of intracavitary treatment were 2900 rad at the base of the bladder and 2000 rad at the anterior wall of the rectum. The Siemens Gammameter is 6% more sensitive for radium than for cesium within 30 mm of the source and the values were correspondingly corrected. Correction for decreased sensitivity of the instrument with an increase in temperature was also attempted (JOELSSON & BACKSTROM 1969).

The radiation doses in the common and external iliac veins during one course of treatment are given as mean values of the absorbed dose in pairs of LiF detectors and referred to the anatomy of the vessels. Anatomic inequalities necessitate the values from right and left side being presented separately.

The dose varied between 71 and 215 with a mean of 155 rad in the cranial part of the right common iliac vein with values of 138 to 691, mean 344 rad in the caudal portion of the same vein. The lowest value was 246 and the highest 1044, mean 470 rad in the cranial part of the external iliac vein while in the caudal part the variation lay between 292 and 1031, mean 426 rad. The dose in the cranial part of the left common iliac vein was between 78 and 273 with a mean of 158 rad. The dose ranged between 204 and 491, mean 334 rad in the caudal part of the vein. The dose varied between 302 and 699, mean 467 rad in the cranial part of the external iliac vein and in the caudal part of the external iliac vein the corresponding values were 312 to 664 with a mean of 445 rad. The one detector located exactly beneath the inguinal ligament was always treated separately. Dose variation on the right side was 245 to 646, mean 321 rad and on the left side 242 to 465, mean 348 rad. Detectors were sometimes placed in the inferior vena cava and doses averaging about 100 rad were registered. One or two detectors were introduced in 11 patients into the middle part of the common iliac vein on the left side and in 4 patients into the right side. The mean doses amounted to 260 to 280 rad. Detectors were also dependent on anatomic variations put into the middle part of the external iliac vein i.e. 9

Table 3

Individual differences in dose rates between measurements in lithotomy and supine positions as percentages of values obtained with the patient in lithotomy position. Remote afterloading technique

	Difference in dose rate as percentage of lithotomy position value	
	Urinary bladder	Rectum
+ 1		-21
-27		0
+22		-12
+ 2		- 1
-19		+10
+ 0		+14
+ 1		+72
+ 2		- 4
+ 4		+46
-19		+75
+ 7		+12
+13		+94
- 9		+ 11
+ 2		-30
0		- 3
+19		- 1
+ 9		+32
+14		+33
Limits	-27 and +22	-30 and +94
Mean	+ 2	+18
S E	±3.1	±8.1

et coll 1969) Primary radiation treatment consisted of two courses of intra uterine (^{137}Cs , 600 mCi) and intravaginal (^{137}Cs , 600 mCi) applications, three weeks apart. The combined intravaginal and intra uterine applicators were connected to the remote afterloading unit, Cervitron 7S (manufactured by Nucleon). The dose rates at the posterior wall of the bladder and the anterior wall of the rectum, with the treatment sources in position, were measured with the Siemens Gammameter immediately after final fixation of the applicators to the patient. This was done with the patient supine, in which position the dose rates were also measured. This was considered necessary because in a separate study in 18 patients, treated with the applicator combination No. 1 considerable differences

occurred between the dose rates at the anterior wall of the rectum measured in lithotomy and supine positions (Table 3). The range of differences was large with a minimum of -30% and a maximum of $+94\%$ with a mean of $+18\%$. Six of the 18 patients exhibited differences of over $+20\%$ and for 4 of them the difference amounted to more than $+40\%$. The mean difference of $+18\%$ cannot be explained by inaccuracies in measurements, but suggests a true difference between dose rates in the two positions. No such differences were observed in dose rates on the posterior wall of the urinary bladder. For the detailed description of the procedure of dose rate measurement the reader is referred to a previous paper by JOELSSON & BACKSTROM (1969).

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Table 4

The radiation doses in rad (mean values and limits) during one course of intracavitary radiation treatment with the gold screened vaginal applicator of the afterloading unit together with an intra uterine stainless steel sound. Bilateral catheters with thermoluminescence LiF detectors were employed in 8 patients

	Inferior vena cava	Common iliac vein			External iliac vein			Under inguinal ligament
		Cranial	Middle	Caudal	Cranial	Middle	Caudal	
Right side								
Mean	93	155	282	344	470	576	426	371
Limits	44—123	71—215	217—323	138—691	246—1 044	344—1 043	292—1 031	245—646
Left side								
Mean	90	158	263	334	467	507	445	348
Limits	54—134	78—273	165—339	204—491	302—699	357—676	312—664	242—465

detectors (6 patients) on the right and 10 detectors (8 patients) on the left side. On the right side the doses were 344 to 1 043, mean 526 rad, and on the left side 357 to 676, mean 507 rad. The dose contribution from diagnostic roentgen procedures amounted to 2 to 3 rad. It should be emphasized that the accuracy of the thermoluminescence technique was about $\pm 8\%$ (standard deviation as percentage of the mean). A complete list of the dose determinations is given in Table 4.

Discussion

A critical review of the factors influencing the dose distribution in intracavitary radiation treatment of carcinoma of the uterine cervix has been given by NEARY (1943, 1947). He concludes that little fundamental improvement in the distribution of the radiation dose can be expected from any variation of the basic Paris or Stockholm methods. The broad features of dose distribution are similar in most existing techniques. The only way to increase the dose in the regions of spread of disease near the pelvic wall is to increase greatly the irradiator activity in the uterus and the vagina. This makes the protection of the rectum and bladder from overdosage of great importance. NEARY used radium sources centrally in the vagina with platinum screens. More recently FLEMING has introduced a similar central source vaginal applicator utilizing ^{60}Co radiation sources and heavy alloy metal (90 to 97 % tungsten) to shield the rectum (FLEMING & WIERNIA 1963). It appears that the lateral decline of doses is little inferior to that of the Manchester technique (BATES et coll 1968).

The vaginal applicator, which is used in connection with the remote after-

loading unit at Radiumhemmet, is ring shaped. The radiation sources in the form of a source train can be dispersed mechanically to the lateral parts of the vagina. ^{137}Cs with a gamma energy of 0.66 MeV is used and the activity of the sources is 600 mCi corresponding to 240 mg radium. In the latest version of the vaginal applicator 18 carat gold screens are adapted to the anterior and posterior surfaces of the ring. gold flanges are placed between the separate cesium sources and the sintered tungsten absorbers are maintained in the source train. The dose distribution from the vaginal ring itself appears extremely satisfactory but when the intra uterine tube with its central sources is added to the system the dose distribution appears to be less satisfactory.

The individual variation of absorbed doses is in general less marked with the afterloading technique than with the current Stockholm method. The reasons are associated with the conformity in relation to the individual anatomy and the stability during treatment time achieved by attaching applicators to the patients with a bar and corset system. The traction exercised by the flexible tubes connecting the intracavitary sources with the storage container of the afterloading unit might be thought to cause changes in the position of the vaginal and uterine ends of the sources; no such changes were however observed. The ball joint can be locked very firmly which prevents it from acting as a fulcrum on which the lever can turn. In the current Stockholm technique the vaginal applicator is held in position by gauze packing in the vagina only and is not connected to the intra uterine applicator. The fallacy of relying upon the gauze pack as an invariable spacer between the source and the rectum has been commented upon elsewhere (JOELSSON & BACKSTROM 1969).

A striking similarity in doses in the afterloading technique was evident on the right compared to the left side of the pelvis; the highest mean value on the latter being less than 10 % higher than the corresponding value on the right side. In the series of patients studied with thermoluminescence dosimetry during treatment according to the current Stockholm technique a 50 % difference in dosage from one side to the other was occasionally registered.

Doses at the pelvic wall are often given in the literature relative to the dose in the middle of the paracervical triangle. Careful determination of dose rates of clinical significance at the bladder base and anterior rectal wall is always performed at Radiumhemmet. The dose values at these locations are therefore measured doses and are available for a comparison.

The devices for securing the applicators in the afterloading technique interfere however with the introduction and movements of the probe of the Siemens Gammameter. As the ball joint is located in the medial sagittal plane the Gammameter probe will invariably be directed either obliquely or be displaced laterally. This obstacle causes difficulties in finding the small area of maximum

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detectors (6 patients) on the right and 10 detectors (8 patients) on the left side. On the right side the doses were 344 to 1 043, mean 526 rad, and on the left side 357 to 676, mean 507 rad. The dose contribution from diagnostic roentgen procedures amounted to 2 to 3 rad. It should be emphasized that the accuracy of the thermoluminescence technique was about $\pm 8\%$ (standard deviation as percentage of the mean). A complete list of the dose determinations is given in Table 4.

Discussion

A critical review of the factors influencing the dose distribution in intracavitary radiation treatment of carcinoma of the uterine cervix has been given by NEARY (1943, 1947). He concludes that little fundamental improvement in the distribution of the radiation dose can be expected from any variation of the basic Paris or Stockholm methods. The broad features of dose distribution are similar in most existing techniques. The only way to increase the dose in the regions of spread of disease near the pelvic wall is to increase greatly the irradiator activity in the uterus and the vagina. This makes the protection of the rectum and bladder from overdosage of great importance. NEARY used radium sources centrally in the vagina with platinum screens. More recently FLEMING has introduced a similar central source vaginal applicator utilizing ^{60}Co radiation sources and heavy alloy metal (90 to 97% tungsten) to shield the rectum (FLEMING & WIERNIA 1963). It appears that the lateral decline of doses is little inferior to that of the Manchester technique (BATES et coll. 1968).

The vaginal applicator, which is used in connection with the remote after

loading unit at Radiumhemmet, is ring shaped. The radiation sources in the form of a source train can be dispersed mechanically to the lateral parts of the vagina. ^{137}Cs with a gamma energy of 0.66 MeV is used and the activity of the sources is 600 mCi corresponding to 240 mg radium. In the latest version of the vaginal applicator 18 carat gold screens are adapted to the anterior and posterior surfaces of the ring. gold flanges are placed between the separate cesium sources and the sintered tungsten absorbers are maintained in the source train. The dose distribution from the vaginal ring itself appears extremely satisfactory, but when the intra uterine tube with its central sources is added to the system the dose distribution appears to be less satisfactory.

The individual variation of absorbed doses is in general less marked with the afterloading technique than with the current Stockholm method. The reasons are associated with the conformity in relation to the individual anatomy and the stability during treatment time achieved by attaching applicators to the patients with a bar and corset system. The traction exercised by the flexible tubes connecting the intracavitary sources with the storage container of the afterloading unit might be thought to cause changes in the position of the vaginal and uterine ends of the sources; no such changes were however observed. The ball joint can be locked very firmly which prevents it from acting as a fulcrum on which the lever can turn. In the current Stockholm technique the vaginal applicator is held in position by gauze packing in the vagina only and is not connected to the intra uterine applicator. The fallacy of relying upon the gauze pack as an invariable spacer between the source and the rectum has been commented upon elsewhere (JOELSSON & BJÖRSTRÖM 1969).

A striking similarity in doses in the afterloading technique was evident on the right compared to the left side of the pelvis; the highest mean value on the latter being less than 10 % higher than the corresponding value on the right side. In the series of patients studied with thermoluminescence dosimetry during treatment according to the current Stockholm technique a 50 % difference in dosage from one side to the other was occasionally registered.

Doses at the pelvic wall are often given in the literature relative to the dose in the middle of the paracervical triangle. Careful determination of dose rates of clinical significance at the bladder base and anterior rectal wall is always performed at Radiumhemmet. The dose values at these locations are therefore measured doses and are available for a comparison.

The devices for securing the applicators in the afterloading technique interfere however with the introduction and movements of the probe of the Siemens Gammameter. As the ball joint is located in the medial sagittal plane the Gammameter probe will invariably be directed either obliquely or be displaced laterally. This obstacle causes difficulties in finding the small area of maximum

dose rate. The significance may be considerable as the dose rates are measured close to irradiators of high activity where the dose rate gradient is steep. Only about 5 mm displacement of the cadmium sulphide crystal of the Gammameter sound can give rise to differences in dose rates of 30 to 40 %.

It has been demonstrated earlier that the highest doses attained at the pelvic wall during radium irradiation according to the current Stockholm method were 22 % of the bladder dose and 20 % of the rectal dose (JOELSSON *et coll.* 1969). The dose on the pelvic side wall in the present study was 18 % of the bladder dose and 26 % of the rectal dose. This is a reflection of the fact that gold screens adapted to the vaginal applicator considerably reduce the dose rate at the anterior wall of the rectum. At the base of the bladder, however, the contribution from the intra uterine radiation sources dominates because of the angulation of the uterine sound.

Reference doses other than those in the bladder and rectum are not clinically available. A comparison of the absolute figures of doses over one course of treatment is not to be recommended because of the differences in activities, duration of treatment and the use of absorbing metal screens. In the current Stockholm technique the amount of radium in the uterine applicator varies between 53 and 74 mg, and in the vaginal applicator between 60 and 80 mg. The irradiation time is between 25 and 28 hours for each course. With the afterloading technique 600 mCi ^{137}Cs is applied both in the uterus and in the vagina and the treatment time is between 6 and 8 hours. This means that the activity is increased by a factor of 3.6 and the treatment time is decreased by a factor of 3.8. If the data for the relationship between total dose and treatment time, published by LIVERSAGE (1969) on the basis of information given by MITCHELL (1960), McWHIRTER (1936) and COWELL (1938) be taken into account, the treatment time should have been still further reduced. Such a reduction must be weighed against the reduced radiation dose to part of the tumor and surrounding tissue effected by the gold screens. When continuing the comparison between the two series referred to above, it is interesting to find that the mean dose at the anterior wall of the rectum for the patients in the afterloading series was 20 % less than the corresponding dose over one course of treatment by the current Stockholm technique. This figure is in accordance with LIVERSAGE's recommended reduction in the tumor lethal dose upon change of treatment time from 28 to 8 hours.

Even if shielding devices imbedded into the applicators can indeed be used as means of reducing the sharp decline in doses in lateral directions, this would mean that individualization of the treatment would be rendered considerably more difficult. A great number of applicators of different sizes would have to be available.

The use of heavier shields therefore appears to be of doubtful value. The

employment of radiation sources with lower gamma energies e.g. ^{192}Ir would on the other hand change the situation. The effective energy of 17 gamma ray lines is about 380 keV and the half value layer 2.2 mm in lead in comparison with 5.5 mm for ^{137}Cs . These favourable data might outweigh the drawback of a rapid decay with a half life of 75 days.

SUMMARY

Selective shielding with gold screens considerably increased the pelvic dose in relation to the rectal dose in the treatment of carcinoma of the cervix uteri with two different intracavitary afterloading applicator systems. The vaginal part of the present applicator device represents one phase in continued efforts towards optimum dose distribution. Different types and sizes must become available so as to facilitate individualized treatment by the remote afterloading technique.

ZUSAMMENFASSUNG

Es konnte gezeigt werden, dass die elektive Abschirmung mit Goldschirmen die Tiefen-dose im Becken wesentlich verbessert im Vergleich mit der Dose, die das Rektum erhält, wenn man das Cervixcarcinom des Uterus mit zwei verschiedenen Hinterladermodellen von Applikatoren bestrahlt. Der verbesserte in der Scheide liegende Anteil des Applikators bedeutet ein Fortschritt in der besseren Dosenverteilung im Becken. Verschiedene Größen und modifizierte Modelle müssen geschaffen werden, um in jedem individuellen Fall die Hinterladertechnik mittels Fernkontrolle durchzuführen.

RÉSUMÉ

La protection sélective par des écrans en or augmente considérablement la dose pelvienne par rapport à la dose rectale dans le traitement du cancer du col de l'utérus par deux systèmes différents d'applicateurs intracavitaires chargés après leur mise en place. La partie vaginale de ces applicateurs présente un des éléments d'une série continue de perfectionnements en vue d'une distribution de dose optimale. Il faut créer différents types et différentes tailles d'applicateurs pour faciliter l'adaptation à chaque malade du traitement par applicateurs chargés à distance après leur mise en place.

REFERENCES

- BATES T, FLEMING J A C, PAGE V and WILKINSON G. Further experience in treating cervical cancer with a central source vaginal applicator. *Brit J Radiol* 41 (1968) 119.
- COWELL M A C. Research into time factor in radiation therapy. In 15th Annual Report of the British Empire Cancer Campaign 1958, p. 162.
- FLEMING J A C and WILKINSON G. A fixed source vaginal applicator for irradiation of carcinoma of the cervix uteri. *Clin Radiol* 14 (1963) 28.
- GRODSTEIN C W. X-ray attenuation coefficients from 10 KeV to 100 MeV. National Bureau of Standards Circular 583. Washington 1957.

- JOELSSON I and BACKSTROM A Dose rate measurements in bladder and rectum Intracavitary radiation of carcinoma of the uterine cervix *Acta radiol Ther Phys Biol* 8 (1969), 343
- — DIEHL J and LAGERGREN C Dose distribution from intracavitary radium and supplementary external irradiation with regard to topography of lymph nodes in carcinoma of the uterine cervix *Acta radiol Ther Phys Biol* 9 (1970) 33
- KOTTMEIER H L and WALSTAM R Cs^{137} as a radiation source for intracavitary gynecological application In *The Swedish Cancer Society, Yearbook 3*, p 397 Edited by H Bergstrand Almqvist & Wiksell Stockholm 1962
- LARSSON I, LIDÉN K and STARFELT N Automatic isodose recorder *Acta radiol Ther Phys Biol* 1 (1963), 29
- LIVERSAGE W E A general formula for equating protracted and acute regimens of radiation *Brit J Radiol* 42 (1969) 432
- McWHIRTER R Investigation to find out the approximate minimal lethal dose for small superficial tumours for varying periods of continuous irradiation In *13th Annual Report of the British Empire Cancer Campaign 1936* p 154
- MAYNEORD W V The significance of the roentgen *Acta Un int Cancer* 2 (1937) 271
- MITCHELL J S Studies in radiotherapeutics Blackwell Oxford 1960
- NEARY G J The physical aspects of intracavitary radium treatment of carcinoma of the cervix uteri *Brit J Radiol* 16 (1943) 225 263
- Physical aspects of intracavitary radium treatment of carcinoma of the cervix uteri *Brit J Radiol* 20 (1947) 454
- THORAEUS R Attenuation of gamma radiation from ^{60}Co ^{137}Cs ^{192}Ir and ^{226}Ra in various materials used in radiotherapy *Acta radiol Ther Phys Biol* 3 (1965) 81
- WALSTAM R Studies on therapeutic short distance and intracavitary gamma beam techniques *Acta radiol* (1965) Suppl No 236

NEUTRON RADIATION PRODUCED BY THE 32 MeV ROENTGEN BEAM OF A MEDICAL BETATRON

by

KAJ ERIK LOFÖREN and ERIK SPRING

Nowadays medical betatrons are used mainly for the production of high energy roentgen rays and most radiotherapeutic treatment is given with the maximum energy of the machine.

Neutron radiation is created when the energy of the roentgen rays exceeds the threshold energy for a (γ, n) reaction; this is unwanted radiation when patients are treated with either roentgen rays or electrons from a betatron. The most important reactions concerned in the irradiation of human tissue are those in ^{12}C , ^{14}N and ^{16}O with the threshold energies 18.72, 10.55 and 15.67 MeV respectively. When the betatron beam strikes heavy materials of the collimating systems or other parts of the machine, neutron radiation is also produced.

Difficulties have been encountered in earlier investigations of the neutron radiation in view of the strong electron or roentgen radiation that affects the measurements. These difficulties can be overcome by application of a detection method that is not disturbed by other radiations. The solid state track detectors used in this study are suitable in this respect.

The purpose of this investigation was to develop a solid state track detector,

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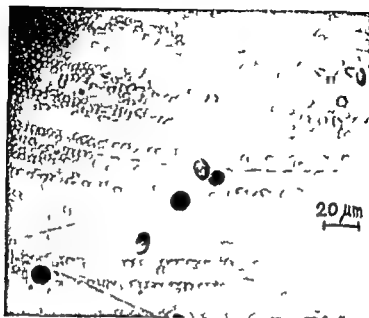


Fig 1 Etched tracks in soda lime silica glass

intended especially for measurement of the neutron contamination in the beam of the medical betatron and in the neighbourhood of the machine. Furthermore, a great deal of care was given to the determination of the neutron radiation to the patient under treatment.

Solid state track detectors When heavy particles such as fission fragments or atomic nuclei, traverse certain insulating solids they leave trails of radiation damage, which become evident as tracks when examined under a transmission electron microscope (FIEISCHER et coll 1965). The trails of radiation damage are chemically attacked much more rapidly in an etching solution than is the undamaged part of the solid (PRICE & WALKER 1962). After the etching procedure, the tracks are visible under an optical microscope: they appear black in normal bright field illumination and white when viewed on a dark field.

The solid state detector also provides a possibility of detecting neutrons by allowing them to traverse a neutron fissionable material which is put close to a track detecting solid in which the resultant fission fragments cause damage.

The solid state track detector is usable as a radiation dosimeter, as the track intensity, that is, the number of tracks per unit area is a measure of the radiation. Consequently the detector can be calibrated by the aid of known neutron sources.

After experiments with a number of different materials (e.g. biotite muscovite polycarbonate resin) glass was found to be the best material for these measurements. The detector material consisted of an ordinary soda lime silica glass in which uranium oxide had been mixed to the amount of 1.5% uranium. The detectors were prepared at the Nuntajarvi Notsjo Glass Wartsila Corporation. The detectors measured 17 mm \times 30 mm \times 2 mm.

The etching was effected in 38 to 40% hydrofluoric acid for 12 seconds. The temperature of the etching solution was 65°C. A criterion in the determination of the etching parameters was that the tracks should be clearly visible under an optical microscope with a magnification of \times 900. In counting the tracks only those with a sharp base were accepted. An example of the tracks is reproduced in Fig. 1.

Detection method. The following formula is valid for the number of fissions k :

$$k = \sigma_f N x \Phi \quad (1)$$

where σ_f is the fission cross section, N is the number of fissionable atoms within depth x of the detector material, τ is the mean path of a fission fragment for producing a track visible after etching, and Φ is the total neutron fluence.

The track density T , i.e. the number of tracks per cm^2 , is given by the formula

$$T = \beta k = \beta N x \sigma_f \Phi = F \sigma_f \Phi \quad (2)$$

where β is the efficiency of the detector and F ($= \beta N x$) in this paper is called the detector constant (which is mainly dependent upon the etching parameters, reagent, time, temperature and type of glass). It has been found that the efficiency of glass detectors is about 0.40 (DEBEAUMAIS et al. 1964).

The detector constant can be determined experimentally by irradiation of the detectors by monoenergetic neutrons of known energy. By determination of the track density the F value is obtained from formula (2). When the neutron energy varies the effective cross-section σ_f and the integrated fluence Φ have to be determined from the energy distribution of the neutrons.

The detectors were calibrated against two Am-Be sources (Department of Physics, University of Helsinki and Institute of Radiation Physics, Helsinki). With the neutron source placed in water, the neutrons are in thermodynamical equilibrium with the water when the distance is greater than 2 cm, and it can be assumed that the spectrum consists of a Maxwell distribution and a tail, which is $1/E$ -distributed, where E is the neutron energy (BECKurts & WIRTZ 1964). A temperature of 293°K, corresponding to a neutron energy of 0.025 eV, was used in calculation of the Maxwell distribution. The cross-sections for

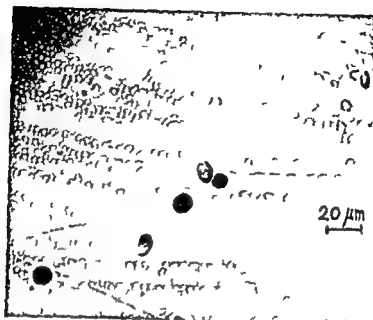


Fig 1 Etched tracks in soda lime silica glass

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The solid state track detector is usable as a radiation dosimeter as the track intensity, that is, the number of tracks per unit area, is a measure of the radiation. Consequently the detector can be calibrated by the aid of known neutron sources.

Table 1

Results of measurements in a water phantom

Depth \bar{z}_{rm} (cm)	\bar{R}_e (rem/n/cm)	\bar{QF} (rem/ rad)	Neutron dose equivalent (mrem/rad roentgen dose*)		Neutron dose (mrad, roentgen dose*)		
			Inside beam	Outside beam	Inside beam	Outside beam	
0.3	116	8.06 ± 0.1	7.6	0.071 ± 0.005	0.011 ± 0.006	0.0028 ± 0.0007	0.0028 ± 0.0008
5.0	225	3.58 ± 0.10	5.0	0.029 ± 0.007	0.024 ± 0.006	0.0058 ± 0.0007	0.0048 ± 0.0002
13.5	55.9	3.35 ± 0.10	4.8	0.023 ± 0.005	0.019 ± 0.005	0.0048 ± 0.0001	0.0040 ± 0.0001
20.5	55.9	3.35 ± 0.10	4.8	0.072 ± 0.005	0.027 ± 0.004	0.0046 ± 0.0001	0.0056 ± 0.0001

*Roentgen dose measured in water at the maximum point of the depth dose curve

$\lambda = (6.9 \pm 0.2) \cdot 10^{17} \text{ a}^{-1}$ (FLEISCHER & PRICE 1964) This implies about 25 fissions per hour in one gram of ^{235}U

The final formula for the neutron fluence is

$$\Phi_m = k T - T, \quad (4)$$

where T corresponds to the track density caused by spontaneous fissions

If photon energies that exceed 5 MeV are used photofission reactions are also possible although the cross sections for these reactions are so low that the reactions do not disturb the measurements. An estimate indicates that only about 3 per thousand of the tracks originate from these reactions.

Finally when the neutron fluence is determined the corresponding dose equivalent DF (rem) can be calculated

$$DE = \bar{R}_e \Phi_m \quad (5)$$

and the corresponding dose D (rad)

$$D = \frac{DF}{QF} = \frac{\bar{R}_e \Phi_m}{QF} \quad (6)$$

where effective values of the conversion factor R_e (rem/(neutrons/cm)) and the quality factor QF must be used in view of variations in the neutron energy. The \bar{R}_e calculations were effected with the neutron energy spectra determined and the R_e -values based upon information given in NBS Handbook No. 63 (1957). The effective quality factor was calculated with the aid of values given in this handbook as well as in ICRP No. 4 (1964).

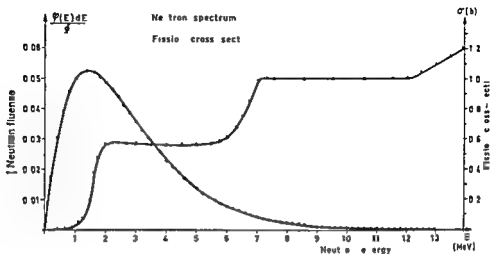


Fig 2 Spectrum of the neutron radiation from the betatron and cross sections for natural uranium calculated with the aid of the values published by SCHMIDT (1962)

natural uranium (Fig 2) have been calculated with the aid of the values published by SCHMIDT (1962) and HALPERN (1959)

When the integrated neutron fluence Φ and the effective cross-section $\bar{\sigma}_f$ are known, the detector constant F is determinable by the application of formula (2). The numerical integrations were made in accordance with the Weddles rule (MARGENAU & MURPHY 1964), and $F = (0.66 \pm 0.10)$ tracks/(neutrons barn) was obtained.

It is now possible to determine the total neutron fluence Φ_m measured with the detectors concerned. The following formula is valid:

$$\Phi_m = \frac{T_m}{\bar{\sigma}_{fm} F} = k T_m \quad (3)$$

where $\bar{\sigma}_{fm}$ is the effective cross section for the neutron fluence under investigation, F the detector constant obtained above, T , the measured track density, and $k = 1/\bar{\sigma}_{fm} F$.

The effective cross-section $\bar{\sigma}_{fm}$ must be determined separately for every measurement if the measuring conditions are changed. The calculations in this study were based upon values reported by SCHMIDT (1962), and the cross sections obtained are given in Table 1.

In the application of formula (3) a correction for spontaneous fission in ^{235}U must be taken into consideration, as the spontaneous fission decay constant

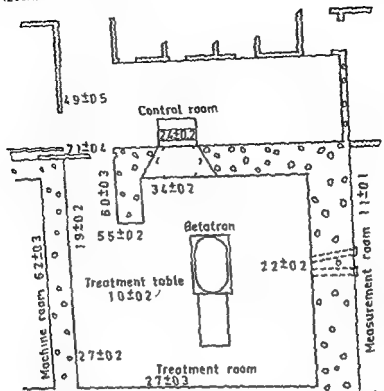


Fig. 4. Schematic figure illustrating the neutron dose equivalents in mSv/h measured at different points in the surroundings of the betatron. Asterisk indicates measurement under the treatment table.

the same value was obtained both inside and outside the beam. The measurements were made up to a distance of 7 cm outside the field used. The values were higher at the central ray: $0.039 \pm 0.006 \text{ mrem/R}$ and $(5.1 \pm 0.7) \cdot 10^3 \text{ (n cm}^2\text{)/R}$ respectively.

Measurements in a water phantom. The neutron dose in water — tissue equivalent material — consists of the neutrons measured earlier together with the neutrons originating from (γ, n) reactions in tissue, mostly from the $^{18}\text{O}(\gamma, n)^{15}\text{O}^*$ reactions.

The neutron spectrum used for the calculation of $\bar{\sigma}_{fn}$, \bar{R}_c and \bar{QF} was a sum of a Maxwell distribution and a neutron spectrum of the $^{18}\text{O}(\gamma, n)^{15}\text{O}^*$ reaction presented by Fuchs et al. (1965). The neutron spectrum varies for different depths in the water (Beckurts & Wirtz 1964). When the depth exceeds 10 cm it can be assumed constant.

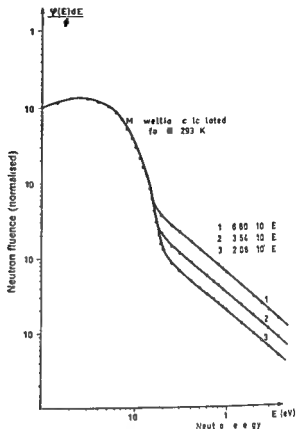


Fig 3 Calculated neutron spectra in water at (1) 5.5 cm (2) 13.5 cm and 20.5 cm depths and (3) at a distance of 7.0 cm from an Am Be source

Measurements

All the measurements were made with $SSD = 80$ cm, field $10 \text{ cm} \times 10 \text{ cm}$ and a roentgen energy of 32 MeV. In every single measurement, the roentgen dose corresponded to a dose of 860 rad measured in water at the maximum point of the depth dose curve.

Measurements in air The neutrons originate from (γ, n) reactions in the betatron collimator and other heavy material of the machine. The energy spectrum must be calculated theoretically (BLATT & WEISSKOPF 1952). It is assumed that the spectrum is Maxwell distributed with a temperature of $O = 1.4 \text{ MeV}$ ($\pm 7\%$), where the errors shown are the possible maximum deviations, by reason of the approximations made in the calculations (Fig 2). The effective cross section $\bar{\sigma} = 86.9 \text{ mb}$, $\bar{R}_e = 7.66 \cdot 10^9 \text{ rem}/(\text{n cm})$, and $\bar{QF} = 7.4$ were determined.

An average value of $0.014 \pm 0.006 \text{ mrem/R}$ was obtained with the exposure measured at the central ray, 80 cm from the anticathode. This corresponds to a neutron fluence of $(1.8 \pm 0.7) \cdot 10^3 (\text{n/cm})/\text{R}$. It should be observed that

measurement are indicated in the figure. Most of the results are of the order of the allowed maximum dose equivalent 25 mrem/h, which of course also includes all other types of irradiation. No one is allowed to stay in the neighbourhood of the door of the treatment room or in the machine room during the irradiation.

Discussion

The results obtained in this study are compared in Table 2 with those published earlier. The differences in the results are difficult to explain. Of course all the other measurements are based upon activation analysis and at least some of them are influenced by the strong roentgen beta or proton radiation present during the measurements. The solid state track detector should not be affected by radiation other than neutron.

The results presented here have moreover been checked with a BF_3 proportional counter (ANDERSSON & BRAUN 1964) developed by AB Atomenergi and made by 20th Century Electronics. With this counter 115 ± 5 mrem/h was obtained in air at the central ray 80 cm from the anticathode. The corresponding value deduced from the measurements with the detectors employed was 98 ± 16 mrem/h. Measurements made behind 8.2 cm water gave 80 ± 10 mrem/h (proportional counter) and 75 ± 18 mrem/h (SST detectors). The SSTD measurements are also in agreement with scintillator counter (boron enriched scintillator) measurements made by RYTÖÄ (1962).

The dose equivalent to the patient caused by the neutron radiation is 0.11–0.16 rem during a treatment of 6000 rad. This is a small value (0.0037–0.0063 rad $QF=30$) even for the eyes which are sensitive to neutron radiation.

Acknowledgement

The authors wish to thank the Wartsila Corporation Nuutajarvi Notsjo Glass for preparing the detectors used in this study.

SUMMARY

Solid state track detectors consisting of 1% uranium soda lime silica glass have been used for investigation of the neutron fluence and the corresponding neutron doses and dose equivalents inside, outside and in the neighbourhood of the roentgen beam of the medical betatron.

ZUSAMMENFASSUNG

Kernspurendetektoren aus festem Material bestehend aus 1% igen Uran Soda Kalk Niesel Glas wurden für Untersuchung der Flussdichte von Neutronen und der entsprechenden Neutronendosen und der Dosis Äquivalente innerhalb, ausserhalb und in der Nähe des Strahlenganges eines medizinischen Betatrons verwendet.

Table 2

Measurements of neutron radiation in the roentgen ray beam of a betatron

Investigation	Energy (MeV)	Neutron dose equivalent per roentgen exposure (mrem/R)	Neutron fluence ((n/cm ²)/R)	Betatron
WAFFLER	31	2.8	$1.3 \cdot 10^4$ at 1 m	BBC-31 Medical
LAUCHLIN (1951)	23	3.0	$4.2 \cdot 10^4$ at 0.84 m	University of Illinois Medical
POHLIT (1960)	31	2.1	$3.1 \cdot 10^4$	BBC 31 research
FROST & MICHEL (1964)	34	15	$1.7 \cdot 10^4$ at 1 m	Asklepitron
BRENNER (1965)	32	1.0	$1.3 \cdot 1.7 \cdot 10^4$ at 1 m	Asklepitron
Present	32	0.039	$5.1 \cdot 10^4$ at 0.8 m	Asklepitron

Asterisk denotes calculated with an effective value of $R_0 = 7.66 \cdot 10^{-6}$ rem/(n/cm²)

The spectrum at a depth of 0.3 cm is assumed to be a Maxwell distribution, with $O = 1.4$ MeV and an added tail of photoneutrons. For depths of 5.5, 13.5 and 20.5 cm, the distribution consists of a Maxwell distribution ($\theta = 0.025$ eV $\approx 293^\circ$ K), and an $1/E$ tail. These spectra are shown in Fig. 3, together with the spectrum used for the Am-Be source in water. The influence of neutrons from $^{16}\text{O}(n, ^{13}\text{O}^*)$ reactions is accounted for in the $1/E$ tail, since these neutrons have not reached thermodynamical equilibrium with the water.

All the values obtained for $\bar{\sigma}_{1n}$, \bar{R}_c , \bar{QF} and the measurements made with the solid state detectors, are contained in Table 1. The neutron doses and dose equivalents correspond to a roentgen dose of 1 rad at the maximum point of the depth dose curve, i.e. at a depth of 5 cm in water.

Table 1 illustrates that the neutron radiation is inclined to be similar within and outside the beam. It can be said that on the average the neutron dose equivalent is 0.0045 ± 0.0004 mrem per 1 rad roentgen dose at the dose maximum. It must be emphasized that the measurements are made only to a depth of 20.5 cm in water, and to a distance of 7 cm outside the beam, i.e. the irradiation field.

Measurements during treatment. Detectors were placed at different points in and outside the treatment room. A schematic figure of the room, the betatron, and the surroundings is reproduced in Fig. 4. The detectors were kept there for three weeks, with 40 hours treatment time per week. The results of

SURVIVAL CHARACTERISTICS OF MAMMALIAN CELL LINES AFTER SINGLE OR MULTIPLE EXPOSURES TO ROENTGEN RADIATION UNDER OXIC OR ANOXIC CONDITIONS

by

Bo LITTEBRAND

Much information has accumulated during the past decade on the radiation survival of a great variety of mammalian cell lines with the clonogenic ability of the cell as an index (LILJED & WHITMORE 1967). Most of the observations concern the survival characteristics after irradiation under aerobic conditions. Few investigations have treated the problem of the dose effect relationship with irradiation in hypoxia or in a total absence of oxygen. The data in the relatively few publications have often been inconsistent. Attempts to resolve the contradictions (e.g. at the 1st Gray Memorial Meeting in London 1967) have met with only partial success. The differences in the biologic materials combined with the differences in the experimental procedures especially those that concern control of hypoxic conditions present serious obstacles to a conjoint interpretation of the available data.

The investigations to be described were conducted with the aim of comparing the reactions of different cell lines to oxidic and anoxic roentgen radiation under

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RÉSUMÉ

Des détecteurs à l'état solide faits de verre de silicate de soude et de chaux à 1 % d'uranium ont été utilisés pour étudier la fluence des neutrons et les doses correspondantes des neutrons ainsi que les doses équivalentes à l'intérieur et à l'extérieur et dans le voisinage du faisceau d'un betatron médical.

REFERENCES

- ANDERSSON I Ö and BRAUN J A neutron rem counter *Nukleonik* 6 (1964) 237
 BECKURTS K H and WIRTZ K Neutron physics Springer Verlag Würzburg 1964
 BLATT J M and WEISSKOPF V I Theoretical nuclear physics John Wiley & Sons New York 1952
 BRENNER M Fast neutron measurements by means of threshold detectors in the presence of 32 MeV X rays *Soc Sci Techn Comm Phys Math* 31 (1965) No 3
 DEBEAUVAIS M MAURITTE M MORY J and WALKER R Registration of fission fragment tracks in several substances and their use in neutron detection *Int J appl Radiat* 15 (1964), 289
 FLEISCHER R L and PRICE P B Decay constant for spontaneous fission of ^{238}U *Phys Rev* 133 B (1964) 63
 — — and WALKER R M Tracks of charged particles in solids *Science* 149 (1965) 383
 FROST D and MICHEL I Über die zusätzliche Dosiskomponente durch Neutronen bei der Therapie mit schnellen Elektronen sowie mit ultraharten Röntgenstrahlen *Strahlen therapie* 124 (1964) 321
 FUCHS H HAAC D LINDENBERGER K H und MEYER BERKHOUT U Die Spektren der Photoneutronen aus den Reaktionen $^{12}\text{C}(\gamma, n)^{11}\text{C}$ und $^{14}\text{O}(\gamma, n)^{14}\text{O}^*$ *Naturforsch* 17a (1962) 439
 HALPERN I Nuclear fission *Ann Rev nuclear Sci* 9 (1959) 245
 ICRP Publ No 4 see Protection against electromagnetic radiation above 3 MeV and electrons neutrons and protons
 LAUCHLIN J S Use of a 23 MeV medical betatron *Nucleonics* 8 (1951) No 4 p 5
 MARGENAU H and MURPHY G M The mathematics of physics and chemistry Second edition D van Nostrand Co Princeton 1964
 NBS Handbook No 63 see Protection against neutron radiation up to 30 MeV
 POHLIT W Die Messung der Neutronenstreustrahlung an einem 35 MeV Betatron *Strahlen therapie* 113 (1960) 469
 PRICE P B and WALKER R M Observations of charged particle tracks in solids *J appl Phys* 33 (1962) 3400
 PROTECTION AGAINST ELECTROMAGNETIC RADIATION ABOVE 3 MEV AND ELECTRONS NEUTRONS AND PROTONS Report of Committee IV (1953—1959) ICRP Publ No 4 Pergamon Press Ltd Glasgow 1964
 PROTECTION AGAINST NEUTRON RADIATION UP TO 30 MEV NBS Handbook No 63 National Bureau of Standards U S Department of Commerce Washington 1957
 RYTILÄ A Inspection certificate Institute of Radiation Physics Helsinki 1962
 SCHMIDT J J Neutron cross sections for fast reactor materials P II Tables Institut für Neutronenphysik und Reaktortechnik Kernforschungszentrum Karlsruhe KFK 120 (LANDC E 35U) Karlsruhe 1962
 WAFFLER H Cited by BRENNER

the strain was discontinued. New cultures were established from earlier samples which were preserved frozen in a tumor bank.

Irradiation Radiation was generated by a Siemens roentgen unit at 190 kV and 15 mA. The radiation was filtered by 1.5 mm Al inherent filtration and a 1.0 mm Al additional filter, half value layer 11.95 mm Cu. The dose rate was 235 R/minute at the bottom of the culture dishes which were always placed at a distance of 43 cm from focus. The dose delivered was measured by a Philips integrating dosimeter. Pyrex petri dishes were used in all the tests. The correction factor for back scatter established from survival measurements of Chinese hamster cells while irradiated on glass versus plastic was 1.33 ± 0.05 .

Experimental procedure Suspensions of monodispersed cells were prepared from 6 to 8 days old cultures growing in a non confluent monolayer on the surface of stoppered milk dilution bottles. The cells were readily dissociated by treatment with a 0.5% trypan solution at a temperature of 37°C for 10 minutes. After establishing the cell concentration in a haemocytometer, an appropriate number of cells was explanted in pyrex dishes of 6 cm diameter containing 4 to 6 ml of nutrient medium. The number of cells was chosen so that about 100 cells were expected to survive exposure to a particular roentgen dose. The dishes were incubated for 3 to 4 hours at 37°C in a moist atmosphere with 5% carbon dioxide in air to maintain a pH of 7.0 to 7.2. After incubation, the cells were irradiated and reincubated for 10 days with two medium changes: the first taking place 2 to 3 and the second 6 to 7 days after irradiation. The clones that developed were fixed and stained in situ. Those that comprised more than 50 cells were counted and taken to represent the surviving fraction of the explanted population. Mean values were routinely calculated from three (irradiated cultures) or six (unirradiated controls) replicate dishes. The fraction of cells surviving irradiation was expressed as the percentage of the unirradiated controls in the same experiment. The plating efficiency of unirradiated controls was defined as the percentage of 100 to 200 cells that grew into macroscopic colonies in 10 days.

Roentgen irradiation was administered at a temperature of about 27°C in an air tight plastic chamber of 2 liter volume. The chamber had two orifices of 6 mm diameter for the in- and outlet of gases. The lids of the culture dishes were removed and the nutrient medium withdrawn to such an extent that no more than 1 ml fluid to cover the cells was left. The chamber was filled with either oxygen or argon both supplemented with 1.25% carbon dioxide, the flow rate being adjusted to 6 liter/minute. The gases passed through pre-equilibrated water warmed to a temperature of 38°C. Argon with an oxygen contamination less than 2 ppm was used and led to the chamber through a system of stainless

identical experimental conditions. In view of recent observations (CHAPMAN et coll 1968, LITTBAND & REVEZ 1969) particular attention was paid to the control of the oxygen concentration at a level previously thought to be of minor importance in radiobiologic experiments. The design of the experiments received special attention to ensure that the data should be suitable for statistical analysis. A closer knowledge of the cellular reactions to hypoxic or anoxic irradiations may be both of theoretical and practical interest. It may help to throw further light on the mechanism of the biologic actions of irradiations and to develop more effective plans for the treatment of neoplasms by more effectively controlling their portions with a deficient oxygen supply.

Materials and Methods

Cell cultures Cultures of Chinese hamster cells (V 79 379 A), an Ehrlich ascites tumor (LLD), and three substrains of the latter, denoted ELT, SELD clone a and SLID clone g, were used. The isolation of the clones and their characteristics have already been described (HAUSCHKA et coll 1957, REVEZ, GLAS & HILDING 1963b). The LLD substrain has a duplicated, i.e. hypertetraploid, chromosome set in relation to ELD and is also characterized by an increased radioresistance under certain *in vivo* conditions, as compared to the original cell strain (GLAS & REVEZ 1963). The cells in clone a and clone g have chromosome sets in the same poidy region as ELD but differ in their sensitivity to radiation *in vivo*, clone a being as sensitive as LLD and clone g less sensitive (REVEZ et coll 1963b).

The Chinese hamster cells were received from Dr Elkind of Bethesda in 1964 and were already adapted to growth in culture. The cultures of ELD and its substrains were established from ascites material grown *in vivo*. Ascites of a particular strain freshly withdrawn was dispensed in amounts of 0.1 ml in stoppered Pyrex milk dilution bottles of 120 ml volume. The culture medium consisted of Eagle's medium in Earle's solution (EAGLE 1959) supplemented with 15% fetal calf serum and antibiotics. After a period varying from 7 to 18 days, multiple foci of vigorously growing colonies became visible, firmly attached to the bottom of the culture flasks. The colonies were detached and dispersed by gentle trypsin treatment, the cells being cultivated in serial passages by transfer of 10^4 cells at weekly intervals and by medium changes every second or third day. In order to protect against development of new substrains, prone to occur in prolonged serial cultures *in vitro* (CAILLIAU & COSTA 1961, NIFLSEN 1967), the chromosomal constitution of the cell population was consequently examined at intervals of about ten successive passages as described previously (REVEZ et coll 1963b) and whenever changes were noted the propagation of

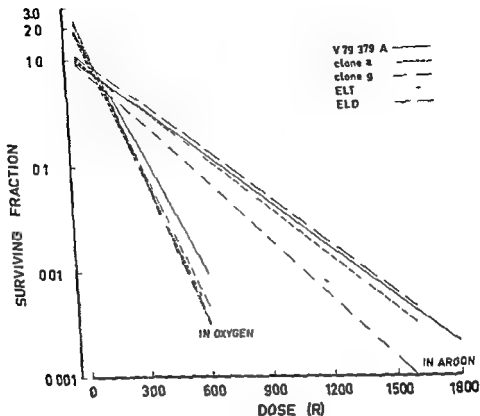


Fig 2 Survival regression of the cell lines after exposure to roentgen radiation in argon or oxygen. The numerical value of the regression parameters and their S.E. is presented in Table 1.

ilarity between the plating efficiency of the cells that were exposed in either argon or oxygen indicates that the difference in the gas phase does not affect the survival of unirradiated cells to any appreciable extent under the conditions used (cf Tables 3, 4 and 6).

Statistical considerations. A main consideration in the design of the experiments concerned the suitability of the data expected for a statistical evaluation of their significance. In determining the variance of the slope constant ($1/D_0$) and the extrapolation number (n) the statistical requirement for independent observations is satisfied if several repeat tests of the survival regression are performed; then the $1/D_0$ and n of each survival curve may be regarded as a single observation. The statistical degrees of freedom are determined solely by the number of

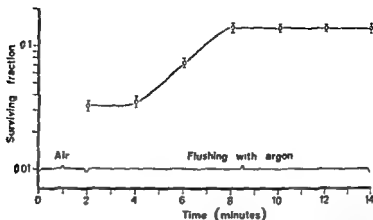


Fig 1 Survival of Chinese hamster cells exposed to 500 R during different periods in the course of a 12 min period of flushing of the irradiation chamber with argon. The results are from 6 repeat tests. The points with S.E. stand at the end of the 2 min irradiation period. The gas flow was 6 liter/minute and the chamber had a 2 liter volume.

steel and glass tubes with joints sealed impermeably to oxygen from the atmosphere. The gas outflowing from the radiation chamber passed a water lock and was routinely monitored by an Elcoflux oxygen analyser (Dr Thiedig & Co KG, Berlin).

The flushing period of the chamber was 2 minutes in the early tests, and 14 minutes in the final tests. The irradiation period varied with different doses and was adjusted to terminate at the end of the flushing period and never started before 6 minutes of flushing.

The survival of Chinese hamster cells treated with 500 R at different times in the course of a 12 min flushing of the irradiation chamber with argon is illustrated in Fig 1. For flushing times of 4 minutes or longer the survival was always similar, indicating that equilibration between the gas phase and the liquid phase that surrounded the cells was complete within 4 minutes. This finding is in agreement with theoretical calculations discussed elsewhere (LITTBAND & REVESZ 1969).

After radiation exposure the dishes were removed from the chamber, refilled with 5 ml fresh medium and incubated in air and carbon dioxide. The exposure procedure with multiple irradiations was repeated after various time intervals. Unirradiated control cultures were sham irradiated under the same conditions as the others. The cultures which received a single exposure dose and belonged to the same experimental series in which the effect of the same dose split into fractions was studied were also sham irradiated at appropriate intervals. The simi-

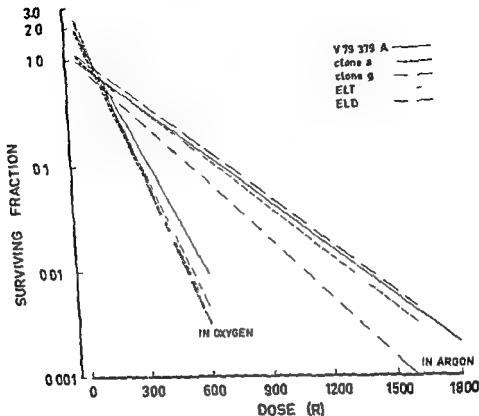


Fig. 2 Survival regression of the cell lines after exposure to roentgen radiation in argon or oxygen. The numerical value of the regression parameters and their SE is presented in Table 1.

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Table 1

Regression parameters of the radiation survival of different cell lines as established by least square analysis of data in repeated tests — Two to four different roentgen doses were used to establish the survival in each test

Cell line	Gas phase during radiation exposure	Plating efficiency	Number repeat tests	Total number surviving fractions determined*	Slope constant of the survival regression Mean \pm S F (%log units/100 R)	D_0 (R)**	Extrapolation number***	
		Mean \pm S F (%)					Mean \pm S F (%log units)	Arithmetic value of mean
I I D	Argon	76 \pm 5	17	36	-0.429 \pm 0.014	233	-0.020 \pm 0.143	0.98
	Oxygen	83 \pm 6	7	20	-1.062 \pm 0.031	94	0.697 \pm 0.158	2.00
F I F	Argon	35 \pm 2	18	52	-0.362 \pm 0.009	276	0.021 \pm 0.080	1.02
	Oxygen	41 \pm 6	7	23	-1.025 \pm 0.032	98	0.780 \pm 0.174	2.18
SELD clone a	Argon	41 \pm 3	20	54	-0.427 \pm 0.011	234	0.227 \pm 0.170	1.26
	Oxygen	41 \pm 2	16	48	-1.048 \pm 0.033	95	0.740 \pm 0.078	2.10
SF I D clone g	Argon	48 \pm 4	24	68	-0.340 \pm 0.016	294	0.030 \pm 0.225	1.03
	Oxygen	31 \pm 1	13	38	-1.087 \pm 0.026	92	0.833 \pm 0.110	2.30
Chinese hamster cells	Argon	76 \pm 4	16	59	-0.354 \pm 0.009	282	0.037 \pm 0.083	1.04
	Oxygen	64 \pm 9	6	18	-0.885 \pm 0.020	113	0.632 \pm 0.076	1.88

*The surviving fraction was determined from the mean value in three irradiated and six control replicate cultures

**Reciprocal of the slope constant indicating the increment of radiation dose which reduces the surviving fraction to 37 %

***The intersection of the statistical regression line with the ordinate at zero dose

separate survival curves regardless of the number of survival determinations which build up any of the curves. The survival determinations pertaining to one individual survival curve are dependent upon a common control, the same particular conditions regarding the quality of the medium, pH and temperature variations which may bias all the observations within a test and do not satisfy, therefore, the requirement for independent data.

These considerations led us to perform a series of at least five repeat tests for determining the parameters of the survival regression of a particular cell line after irradiation. In each test, the survival is in general established after exposure of cells in replicate dishes to two or three different roentgen doses. These are of such a magnitude that the dose-effect relationship can be assumed

Table 2

The ratio and its standard error between the slope constant of theoxic and anoxic survival regressions of the five cell lines tested — Data presented in table 1 were used for calculations — S.E. was approximated by formula (1) given in the text

Cell line	Ratio between the slope constants of the oxic and anoxic survival regressions Mean \pm S.E.
ELD and sub fractions	
ELD	2.48 \pm 0.11
ELT	2.83 \pm 0.12
SELD clone a	2.45 \pm 0.07
SELD clone g	3.20 \pm 0.12
Chinese hamster cells	2.30 \pm 0.09

to be exponential. The survival data even after such a few exposure doses are obviously sufficient for the statistical determination of a linear survival regression by least square analysis. Since the confidence limit of the regression line is inversely related to the difference between the exposure doses (PORTER 1963) one of the doses is chosen, as a routine, closest to a possible shoulder region of the expected survival curve, and another dose to give the minimum survival (in the range 10^{-3}) still detectable by our method with sufficient accuracy. A third dose with a magnitude between the two extremes is also given to permit the control of the linearity of the survival regression. The series of slope constants and extrapolation numbers obtained in repeat tests are then used for the calculation of the mean and the variance of these survival parameters and for the evaluation of their statistical significance.

Results

Single exposures. In a series of repeat tests cultures of Chinese hamster cells, ELD and its derivatives ELT, SELD clone a and clone g were exposed to radiation in the dose range 200–600 R in oxygen or 800–1800 R in argon.

Assuming an exponential decrease of the survival in the dose ranges used (ELAND & WHITMORE 1967) the survival regression was determined by least square analysis in each test and the mean regression of the different test groups calculated. The results are illustrated in Fig. 2. The numerical value of the regression parameters are summarized in Table 1.

The reciprocal of the slope constant of the survival regressions (D_1) indicating the dose that reduces survival to 37 per cent gave values between 92 to 98 R.

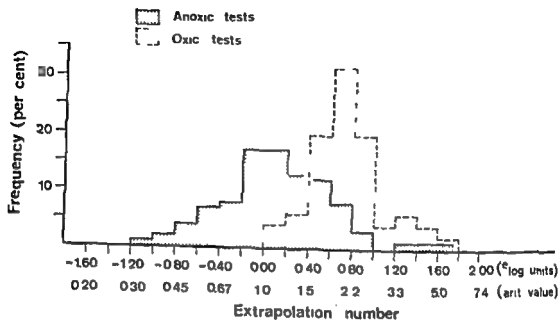


Fig. 3 Frequency distribution of the extrapolation number of the survival regressions calculated for each of the 49 oxic and 95 anoxic tests presented in Table 1

when irradiation of ELD and its substrains was made in oxygen, and between 233 to 294 R when these cell strains were exposed to radiation in argon. The differences between the slope constants of the oxic cells were, as found by *t* test analysis, not significant. When treated in argon the slope constant of ELD and clone a differed significantly from ELT and clone g ($p < 0.001$), but the difference between ELD and clone a or ELT and clone g was not significant. The D_0 for Chinese hamster cells was 113 R in oxygen and 282 R in argon.

The intersection of the regression line of the individual tests with the ordinate at zero dose gave extrapolation numbers the log frequency distribution of which did not deviate from a normal distribution (cf. Fig. 3). The means were in the region of 2 after oxic treatments and close to unity after the anoxic treatments. When tests performed with the same cell strain are considered the difference between the mean extrapolation number in oxygen and in argon is significant ($p < 0.001$ or < 0.02).

The ratios between the slope constants of the survival regressions after oxic and anoxic irradiation are presented in Table 2, the data given in Table 1 being used for the calculation. The S.E. of the ratios were approximated by the following formula

$$S_r = \sqrt{\frac{R_o^2}{R_A} \left(\frac{S_o^2}{R_o^2} + \frac{S_A^2}{R_A^2} \right) \left(1 + \frac{95 S_A^2}{R_A^2} \right)} \quad (1)$$

Table 3

Survival of one hundred Chinese hamster cells after exposure to roentgen doses in the range 50–150 R in either the presence or absence of oxygen — The mean plating efficiency was 96 ± 3 and 95 ± 2 per cent in the tests with argon and oxygen respectively

Number of repeat tests	10		10		10		10		10	
	50		75		100		125		150	
Exposure dose (R)										
Gas phase during irradiation exposure	Ar	O ₂	Ar	O ₂	Ar	O ₂	Ar	O ₂	Ar	O ₂
Survival fraction	93.8	97.6	90.6	88.4	87.0	83.6	80.3	80.8	79.1	73.1
Arithmetic value of mean	4.541	4.579	4.506	4.487	4.466	4.476	4.386	4.397	4.371	4.293
Mean \pm S.E.	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
(log units)	0.075	0.053	0.033	0.023	0.015	0.021	0.078	0.072	0.019	0.021
Ratio between surviving fractions in argon and oxygen	0.012		0.074*		0.040		-0.007*		0.078**	
Mean \pm S.E.	\pm		\pm		\pm		\pm		\pm	
(*log units)	0.041		0.042		0.073		0.031		0.024	

P (probability of no difference from zero) > 0.05

P < 0.01

where S_R is the S.E. of the ratio between the respective slope constants, R_0 and R_4 are the means of the slope constant of the survival regression obtained in repeat tests in oxygen and argon and S_1 and S_4 are the S.E. of R_0 and R_4 respectively

In comparing the ratio between theoxic and anoxic slope constants of ELD with the ratios of its sub strains the t test analysis will indicate that ELD or clone a has a ratio with a significantly smaller value than ELT or clone g ($p < 0.001$ or < 0.05). The value for ELT differs significantly from that of clone-g ($p < 0.05$) but the difference between the ratio of ELD and clone is not significant

Effect of doses below 150 R The difference between the extrapolation numbers after oxic and anoxic irradiation implies a crossing of the regression lines. As can be seen from Fig. 2 the crossing occurs in a dose range around 120 R for each cell strain. In this dose range therefore oxygen may not enhance the

Table 4

Survival of Chinese hamster cells after exposure in argon or oxygen to either a single dose of 300 R or to the same dose divided into three equal fractions and separated by a time interval of three hours — During the intervals the cells were incubated in air and carbon dioxide — The data are from 13 repeat tests

Exposure dose (R)	Treatment in oxygen			
	Cells exposed	Plating efficiency Mean \pm S.E. (%)	Surviving fraction	
			Mean \pm S.E. (log units)	Arithmetic value of mean
3 x 100	300	98 \pm 3	3.59 \pm 0.041	34.1
300	300		2.95 \pm 0.049	19.7

radiosensitivity of the cells. After larger doses on the other hand the oxygen enhancement ratio defined by the ratio between the dose in oxygen and in anoxia which produces identical survival will obviously increase (cf Fig 2). It can be calculated (REVESZ & LITTBAND 1967a) that the maximum oxygen enhancement ratio will be defined by the ratio between the slope constant of theoxic and anoxic survival regressions (cf Table 2).

The effect of oxygen was examined in two series of experiments on the survival around and below the dose range where the oxic and anoxic regression lines cross. Chinese hamster cells in one series were exposed to a single radiation dose in the range 50 to 150 R in the presence or absence of oxygen. The results are summarized in Table 3. The presence of oxygen does not seem to exert any major influence on the surviving fraction after exposure to a dose of 125 R or less. The differences between the oxic and the anoxic groups are statistically insignificant. On the other hand, the surviving fraction is significantly smaller ($p < 0.01$) after exposure to 150 R in oxygen than after treatment with the same dose in argon.

The failure of oxygen to enhance cellular radiosensitivity when treatment is given with relatively small radiation doses was put to a further and more rigorous test in another series of experiments. Chinese hamster cells were exposed three times to a dose of 100 R in the presence or absence of oxygen with an interval of 3 hours between the exposures. Cultures treated under identical conditions with a single dose of 300 R and two subsequent sham irradiations were used as particular controls. The results are presented in Table 4. The surviving fractions were of about the same magnitude after triple exposures when either oxygen or

Table 4 (cont.)

Treatment in argon		Ratio between survival fractions in argon and oxygen			
Cells exposed	Plating efficiency Mean \pm S.E. ()	Surviving fraction		Mean \pm S.E. (%log units)	Arithmetic value of mean
		Mean \pm S.E. (%log units)	Arithmetic value of mean		
300	93 \pm 4	3.568 \pm 0.036	35.5	0.039 \pm 0.040	1.04
300		3.536 \pm 0.040	34.3	0.581 \pm 0.043	1.79

argon was the gas phase. On the other hand, when a single treatment was given with the same total dose, the survival was larger by a factor of 1.79 in argon than in oxygen. The survival after treatment with triple doses may also be calculated as 1.78 times larger than after treatment with a single dose in the presence of oxygen. When oxygen was absent, only an insignificant difference was noted in the effect between a single and triple radiation treatment.

Estimations of the oxygen enhancement ratio from the data presented in Table 3 offer further support to the conclusion that oxygen fails to enhance radiosensitivity in treatment with small roentgen doses. Such estimations may be made by calculating the difference between the survival after irradiation with a dose in argon and with another dose in oxygen, and the corresponding ratio between the doses. Table 5 lists the survival ratios that correspond to roentgen doses chosen in such a way that their ratios are between 1 and 2. In considering such dose combinations, the survival appears always to be better afteroxic than after anoxic treatment. The differences have varying degrees of statistical significance. Since an identical survival afteroxic and anoxic treatment would indicate that the oxygen enhancement ratio equals the corresponding dose ratio, it may be concluded from the data in Table 5 that the oxygen enhancement ratio in these experiments is significantly lower than 2 and probably also lower than 1.2.

Split dose ratio underoxic and anoxic conditions. The effect of two exposures to radiation on the survival of ELD and Chinese hamster cells was investigated in relation to the effect of a single exposure to the same total dose combined with a subsequent sham irradiation. The time interval between two treatments

Table II

Survival ratio after oxie and anoxic exposure to roentgen doses chosen in such a way that the dose ratio was between 1 and 2 — Data in Table 3 were used for the calculations

Exposure dose ratio in argon/in oxygen	Survival ratio Surviving fraction after treatment in oxygen/ surviving fraction after treatment in argon (\log units)
$\frac{75}{50} = 1.5$	0.022
$\frac{100}{50} = 2.0$	0.062*
$\frac{100}{75} = 1.3$	0.015
$\frac{125}{75} = 1.7$	0.096
$\frac{150}{75} = 2.0$	0.111**
$\frac{125}{100} = 1.3$	0.041
$\frac{150}{100} = 1.5$	0.056
$\frac{150}{125} = 1.2$	0.022

*P (probability of no difference from zero) < 0.05 **P < 0.001

was 4, 12 or 18 hours. Irradiations were performed either in the presence or absence of oxygen. The results are illustrated in Fig. 4. Independently of cell strain and time interval between treatments the surviving fraction after two exposures was of a similar magnitude as after a single exposure to the equivalent dose when oxygen was absent. Thus, the split dose ratio defined by the ratio between the surviving fractions after a single and split doses was always close to unity. On the other hand, when irradiated in the presence of oxygen, double or treble the number of cells survived after split dose treatment than after corresponding single dose treatment. When the tests, performed with the same cell strain and with identical time interval between the exposures, are considered, the difference between the split dose ratio after oxie and anoxic treatment is significant ($p < 0.001$ or < 0.005).

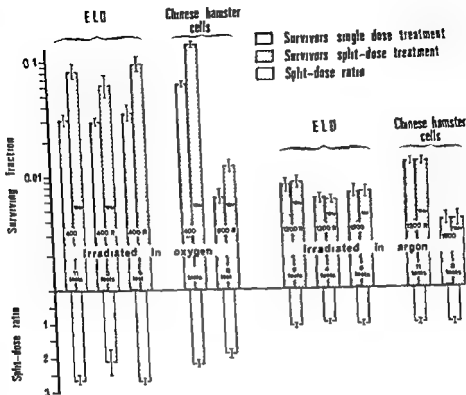


Fig 4 Survival of ELD and Chinese hamster cells after exposure in argon or oxygen to either a single roentgen dose or to same dose delivered in two equal fractions (upper scale) The time interval between the dose fractions was 4 12 and 18 hours as indicated in the respective col mns during which period the cells were incubated in air and carbon dioxide The split dose ratio calculated from the difference between the survival after single and split doses are indicated (lower scale) Each column shows the mean with S.E. The mean plating efficiency varied between 47 to 70 and 91 to 96 per cent in the test with ELD and Chinese hamster cells respectively

A series of five repeat tests was performed to examine the possibility that the difference between the split dose ratios in argon and oxygen might have been due to some particular effect of the argon treatment before irradiation Chinese hamster cells were kept in argon for 15 minutes and within one minute thereafter irradiated in oxygen with either 700 R or 2×350 R at time intervals of 18 hours As a particular control cultures that were kept in oxygen for 5 minutes and subsequently irradiated with the same doses under identical conditions were employed The difference in the pretreatment with the two different gases does not affect either the survival or the split-dose ratio as indicated by the results in Table 6

Table 5

Survival ratio afteroxic and anoxic exposure to roentgen doses chosen in such a way that the dose ratio was between 1 and 2 — Data in Table 3 were used for the calculations

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$\frac{150}{75} = 2.0$	0.111**
$\frac{125}{100} = 1.3$	0.041
$\frac{150}{100} = 1.5$	0.056
$\frac{150}{125} = 1.2$	0.022

*P (probability of no difference from zero) ~ 0.0; **P ~ 0.001

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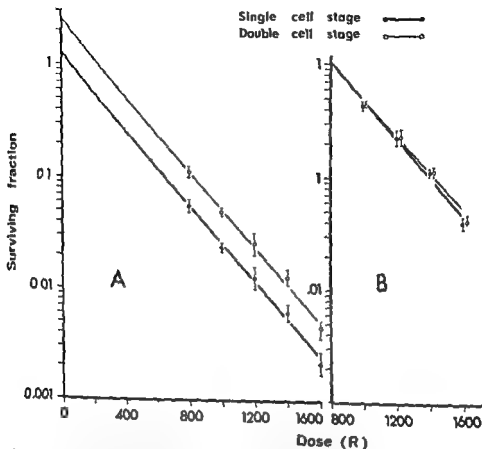


Fig. 5. Survival of Chinese hamster cells after irradiation with increasing doses of roentgen rays in two exposures in argon. The first exposure followed the plating of the cells after a period of 3 hours (single cell stage) or 21 hours (double cell stage) and was in all cases to 800 R. The second exposure followed the first exposure after a period of 18 hours (single cell stage) or 9 hours (double cell stage) to a dose varying from 200 to 800 R. Between exposures the cells were incubated in air and carbon dioxide. Means with 95 per cent confidence limits are shown from 3 repeated tests with a mean plating efficiency of 93 ± 5 per cent. Left part of figure (A) illustrates survival relative to the unirradiated cells; right part (B) the survival relative to the survivors of 800 R treatment.

a single cell in a sister pair $= 2 \times (1 - 0.06) \times 0.06 = 0.1152$. This suggests that the overwhelming majority ($\frac{0.1152}{0.1152 + 0.0036} \approx 97\%$) of the survivors in the population exposed to an initial radiation with 800 R after cell division will consist of single cells and not of sister pairs i.e. the cell multiplicity will be 1 when the second radiation dose is applied.

Table II

Effect of pretreatment with argon or oxygen on the survival of Chinese hamster cells exposed to x-ray irradiation with a single or split doses

Treatment before irradiation	Plating efficiency \pm S.E. (%)	Exposure dose in oxygen (k)	Surviving fraction (%)		Split dose ratio	
			Mean \pm S.E. (log units)	Arithmetic value of mean	Mean \pm S.E. (log units)	Arithmetic value of mean
15 min in argon	83 \pm 5	700	-0.149 \pm 0.115	0.86	0.830 \pm 0.151	2.99
		2 \times 350	0.681 \pm 0.127	1.98		
5 min in oxygen	84 \pm 5	700	-0.173 \pm 0.126	0.84	0.781 \pm 0.100	2.18
		2 \times 350	0.608 \pm 0.130	1.84		

Survival regression after irradiation with split doses The survival regression after split dose treatments was studied by double exposures of the hamster cells to increasing total roentgen doses. The first exposure was always to a dose of 800 R, the second one to a dose varying from 200 to 800 R, all exposures being made in argon. In each of the five repeat tests, the cells were in two different stages of growth when irradiated for the first time. In one case, the first dose was given 3 hours after plating of the cells, followed by the second dose after an interval of 18 hours. In the second case, the cells were irradiated with the first dose 21 hours after plating, and with the next dose after a further interval of 9 hours. These time intervals were chosen from two previous observations. On the one hand, cells that have been irradiated 3 hours after plating, will not divide for 18 hours. On the other hand, if the first irradiation is made 21 hours after plating, the cells will have completed one division and the population will have doubled and consist of sister pairs. In the latter instance, the survivors will not have completed a second division within a further period of 9 hours.

The survival of the irradiated cells in relation to unirradiated controls is illustrated in Fig. 5 A. The numerical value of the survival parameters calculated by least square analysis are presented in Table 7. The slope constraints are almost identical whether the exposure to radiation was made before or after the first division of the cells. In contrast the extrapolation number of the regression line at a zero dose is close to unity when the cells were treated before division and has a value between 2 and 3 when the cells were treated after division.

Considering that the probability of a cell surviving irradiation with 800 R, used as the first dose, is 0.06 (cf. Figs. 2 and 5 A) it can be calculated that the probability of both cells in a sister pair surviving is $0.06 \times 0.06 = 0.0036$ and that of

et coll 1963a) The role of the inherent sulphhydryl in influencing intrinsic radiosensitivity is further supported by the finding that clone a with the same sensitivity as ELD has a thiol concentration of the same magnitude (REVEZ et coll 1963a) The increased radioresistance of ELT with a sulphhydryl concentration of the same magnitude as ELD (CASPERSSON & REVEZ 1963 REVEZ et coll 1963a) can be associated with the duplicated genome of this strain as discussed in other reports (GLAS & REVEZ 1963 REVEZ et coll 1963b REVEZ & LITTBAND 1964) The possible necessity of inactivating a larger number of sensitive sites has probably accounted for the radioresistance in such a case However since the extrapolation number of the ELT survival curve is similar to the extrapolation of ELD whetheroxic or anoxic irradiation was used (cf Fig 2) some other factors in addition to or instead of an increased target number may also play a role The observations of SILINI & HORASEY (1962) and BERRY (1963) on an increased extrapolation number of tetraploid tumors in comparison to diploid ones may not be appropriate in this context The cell lines in their tests were not sublines of each other and the change in the extrapolation number may be associated therefore to some difference between lines other than ploidy

The difference in radiosensitivity demonstrated between the cell strains irradiated in anoxia was shown to be reduced to statistically insignificant variations when the exposures to roentgen radiation was made in oxygen This implies that the oxygen enhancement ratio is different for the different cell strains Statistical analysis of the data indicated that the differences in the oxygen enhancement ratio were significant (Table 3) A reduction of the sensitivity differences in this gas as compared to anaerobic conditions has been previously noted also when the cell strains used in the present tests were investigated *in vivo* (REVEZ et coll 1963b REVEZ et coll 1967) Similar observations were reported by SILINI & HORASEY (1962) in their *in vivo* tests The relationship between the values of oxygen enhancement ratios of diploid and tetraploid cell lines used by these authors (2.7/3.1) was similar to the relationship observed in our case 2.48/2.83

The oxygen effect can apparently mask variation in cellular sensitivity whether due to a difference in ploidy or to the SH content If a proportionately increased sulphhydryl concentration accounts for the relative resistance of some cell lines in anoxia then the disappearance of this resistance in oxygen would be in agreement with the observation of a considerable decrease of the sulphhydryl protection in an oxygenated medium (DUFFY 1963 HOWARD FLANDERS 1960 SHALEK & SMITH 1968) It can also be argued that the correlation between radiosensitivity and ploidy observed when treatment is made in anoxia may imply the principally genetic character of the radiation injury to the cell The decrease or abolition of such a correlation when treatment is made in oxygen suggests the prevalence of

Table 7

Numerical value of the survival parameters in the tests illustrated in Fig. 5

Time interval between plating and 1st irradiation	Time interval between 1st and 2nd irradiation	Survival of irradiated cells relative to unirradiated controls				Survival of irradiated cells relative to survivors of 800 R			
		Slope constant of survival regression Mean \pm S.E. (%log units)	D ₀ (R)	Extrapolation number*		Slope constant of survival regression Mean \pm S.E. (%log units)	D (R)	Extrapolation number**	
				Mean \pm S.F.	Arit. value			Mean \pm S.E.	Arit. value
				(%log units) of mean				(%log units) of mean	
3 hours	18 hours	0.390 \pm 0.018	256	0.232 \pm 0.096	1.26	0.393 \pm 0.027	254	0.031 \pm 0.129	1.03
21 hours	9 hours	0.387 \pm 0.018	259	0.906 \pm 0.076	2.47	0.389 \pm 0.025	257	0.023 \pm 0.120	1.01

*Calculated from the intersection of the statistical regression line with the ordinate at zero dose

**Calculated from the intersection of the statistical regression line with the ordinate at 800 R

In Fig. 5 B the survival of the irradiated cells relative to the survivors of a treatment with 800 R is illustrated. The numerical value of the survival parameters, calculated by least square analysis is presented in Table 7. The slope constants are, again, similar if the data obtained in tests with cells before and after division are considered. However, in this situation the extrapolation numbers are again similar and close to unity in both cases.

Discussion

Slope of survival regression The results indicate that under anoxic conditions significant differences in radiosensitivity exist between cell strains selected from the same original tumor cell population. It may be concluded that in agreement with the wide intercellular variation of different characteristics (HARRIS 1964), neoplastic cell populations may also vary with regard to the radiosensitivity of their cellular components. It may be calculated from the relationship between the slope constants that the sensitivity of clone g and ILT is 24 and 19 per cent less respectively, than the sensitivity of the original ELD. The decreased sensitivity of clone g may be attributed to the protective effect of the increased cellular concentration of low molecular sulphhydryls, which has been estimated to be 24 per cent higher in this clone than in ELD (CASPERSON & REYESZ 1963, REYESZ

et coll 1963a) The role of the inherent sulphhydryls in influencing intrinsic radiosensitivity is further supported by the finding that clone a with the same sensitivity as ELD has a thiol concentration of the same magnitude (REVEZ et coll 1963a) The increased radioresistance of ELT with a sulphhydryl concentration of the same magnitude as ELD (CASPERSSON & REVEZ 1963 REVEZ et coll 1963a) can be associated with the duplicated genome of this strain as discussed in other reports (GLAS & REVEZ 1963 REVEZ et coll 1963b REVEZ & LITTEBRAND 1964) The possible necessity of inactivating a larger number of sensitive sites has probably accounted for the radioresistance in such a case However since the extrapolation number of the ELT survival curve is similar to the extrapolation of ELD whetheroxic or anoxic irradiation was used (cf Fig 2) some other factors in addition to or instead of, an increased target number may also play a role The observations of SILINI & HORNEY (1962) and BERRY (1963) on an increased extrapolation number of tetraploid tumors in comparison to diploid ones may not be appropriate in this context The cell lines in their tests were not sublines of each other and the change in the extrapolation number may be associated therefore to some difference between lines other than ploidy

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The oxygen effect can apparently mask variation in cellular sensitivity whether due to a difference in ploidy or to the SH content If a proportionately increased sulphhydryl concentration accounts for the relative resistance of some cell lines in anoxia then the disappearance of this resistance in oxygen would be in agreement with the observation of a considerable decrease of the sulphhydryl protection in an oxygenated medium DEWEY 1963 HOWARD FLANDERS 1960 SIALEN & SMITH 1968 It can also be argued that the correlation between radiosensitivity and ploidy observed when treatment is made in anoxia may imply the principally genetic character of the radiation injury to the cell The decrease or abolition of such a correlation when treatment is made in oxygen suggests the prevalence of

some non genetic mechanism operative in the presence of this gas and involving structures and systems other than the genetic apparatus alone. This conclusion is in agreement with the postulate based on observations made in experiments with micro organisms that DNA is more heavily involved in killing by roentgen radiation under anoxic as compared to aerobic conditions (AUPER 1963)

Extrapolation numbers The extrapolation number (n) of the survival curves of ELD and its substrains, as well as the Chinese hamster cells, were similar and had values around 2 when irradiations were administered in the presence of oxygen, and values close to unity when the irradiations were given in the absence of the gas. The relatively low value of n of the oxic survival curves may be attributed to the fact that the cells used in the survival tests were derived from 6 to 8 day old cultures, i.e. were in a stationary phase of growth. HAHN (1968) reported that the survival curve of such cells extrapolate to lower values than cells derived from cultures in the phase of exponential growth.

The extrapolation number to unity found throughout in the tests when cells were irradiated under anaerobic conditions, was found (REVESZ & LITTBRAND 1969) to be independent of the growth phase of the cultures from which the cells were derived. This is also indicated by the experiments (cf Table 7) in which the cell population used has completed one division after plating and before irradiation. The cells in such a population may be regarded as having full metabolic activity and being in an early phase of exponential growth. When the survival regression of such cells was established the extrapolation number was unity (cf Fig 5 B), as in the case of cells which were, as a routine, derived from cultures in their plateau phase and which had probably an impaired metabolism.

The difference between the extrapolation numbers when irradiation was made in argon and oxygen as well as the difference in the oxygen enhancement ratio of closely related cell lines suggest that oxygen does not act as a simple, dose modifying factor. It may be inferred that, in addition to a quantitative difference there is also a qualitative difference in the lethal damage caused by irradiation in the presence and absence of oxygen. In view of recent findings that indicate the oxygen dependency of the recovery process it is conceivable that the difference concerns the repair of the radiation damage (LITTBRAND & REVESZ 1969). As an alternative explanation, the extrapolation to unity and a corresponding nearly exponential dose effect relationship may denote that a single damaging event is sufficient to abolish the reproductive capacity of anoxic cells. The dose effect relationship after anoxic treatment with the sparsely ionizing roentgen rays, is indeed similar to that observed after treatment with densely ionizing radiations (BARENDSEN 1962; BARENDSEN et al 1963; DEFRINC & RICE

1962) On the other hand an extrapolation number larger than unity noted after aerobic treatment may be regarded as indicating that more than one damaging event of the same or a different kind (genetic or non genetic) must be accumulated for any lethal effect

The crossing of the extrapolated survival regression of the oxic and anoxic cells (Fig 2) implies that the effect of oxygen in enhancing sensitivity varies with the magnitude of the exposure dose In the dose range before the crossing a negative enhancement i.e. a protecting effect may prevail Experimental evidence for such a protection was not obtained in the tests performed in 100 per cent oxygen with either single exposure doses in the range of 50 to 125 R or with multiple doses of 100 R though this latter treatment may be particularly suitable for discovering small differences in survival The differences between the survival of oxic and anoxic cells were always insignificant and an oxygen enhancement ratio close to unity was indicated With increasing doses after the crossing the oxygen enhancement ratio could be expected to increase from unity toward a maximal value defined by the ratio between the slope constant of the oxic and anoxic survival regressions (REVEZ & LITTBAND 1967a) This expectation received experimental proof in tests in which the survival curve of oxic cells was determined after exposure to doses between 200 and 600 R and that of anoxic cells after exposure to doses between 800 and 1 800 R It is evident that the enhancement of radio-sensitivity by oxygen is a function also of the exposure dose and is therefore more complex than expressed by the formula proposed by HOWARD FLANDERS & ASPLER (1957) A more detailed discussion of this problem has been published elsewhere (LITTBAND & REVEZ 1969)

Recovery and the oxygen effect In considering the relationship between the split dose ratio and the extrapolation number (ELKIND & SURROG 1960) the split dose ratio around 2 and 1 found in the oxic and anoxic experiments respectively is in agreement with the theoretical expectations It has been shown that a summation of survival curves with diverse extrapolation numbers (DITTRICH 1960) or slope constants (LOWERS & TOLMACH 1963) may result in a common composite curve with a decreased extrapolation number The close agreement between extrapolation number and split dose ratio excludes this possibility, i.e. the heterogeneous response of the cell population as an explanation of the findings The recovery observed when cells were pretreated in argon and subsequently irradiated in oxygen (Table 6) also contradicts the argument that such pretreatment is in some way responsible for the impaired recovery when irradiation is made anoxically The data of the different experiments are thus concordant in indicating that recovery requires oxygen The results of experiments in which recovery was studied at varying oxygen tensions strongly suggest that

oxygen is required as the energy source for the recovery process (LITTBRAND & REVE SZ 1969)

Since the cells in the experiments immediately after exposure to radiation in anoxia were put under aerobic conditions, it may also be concluded that post-treatment access to oxygen does not restore recovery. Recovery is, indeed, dependent upon the presence of oxygen during or very shortly after the radiation treatment. It has been argued (LITTBRAND & REVE SZ 1969) that the damage induced by radiation may be recoverable only during a limited time period. If oxygen is present during the critical period, the recovery process may begin and continue whilst its energy requirement is satisfied.

Oxygen may play a dual role in determining the reaction of cells to radiation. On the one hand, it may sensitize the cells in enhancing the radiation damage and, on the other hand, it may protect them in permitting recovery. Apparently, in the dose range around the crossing of the extrapolated survival curves, the protecting effect can wholly compensate for the injury due to the sensitizing effect. With larger doses, the protecting effect has a decreasing significance in relation to the sensitizing effect. The relative importance of the two antagonistic effects has been shown to be dependent also upon the oxygen concentration (REVE SZ & LITTBRAND 1967b, LITTBRAND & REVE SZ 1969). As an example a concentration of $0.5 \mu\text{M}$ oxygen in the medium permits the development of almost maximal recovery while it sensitizes only slightly (LITTBRAND & REVE SZ 1969). This indicates that an oxygen enhancement ratio close to some known maximal value cannot be simply taken as evidence for the prevalence of total anoxic conditions in a certain experimental system.

Present observations in relation to earlier studies. The results presented in this paper are in full agreement with preliminary observations (LITTBRAND & REVE SZ 1961, ROBINSON & REVE SZ 1961). They also confirm the data obtained in experiments in which the effects on radiosensitivity of cysteamine, triethylenemelamine, N-oxyl or oxygen in varying concentrations were studied on radiosensitivity cells irradiated similarly in anoxia or 100 per cent oxygen being used as controls (LITTBRAND & REVE SZ 1969, to be published, REVE SZ & LITTBRAND 1970). The findings reported by PHILLIPS (1968) are also in agreement with our observation on an oxygen dependent extrapolation number. This author irradiated bone marrow cells in vitro in the presence of oxygen or nitrogen. In subsequent in vivo tests with the spleen colony technique the aerobic survival curves extrapolated to 1.5 while the anaerobic curves extrapolated close to unity. In an in vitro system with a long gassing equilibrium time, HALL et al. (1966) found a reduction of the extrapolation number to unity when the radiation survival of HeLa cells was studied after storage in severe nitrogen hypoxia for

36 hours. A 6 hour storage resulted in no reduction of the extrapolation number. In vitro experiments with L cells HUMPHREY et coll (1963) noted a reduction of the extrapolation number from 12 to 3 when cells were irradiated under hypoxic conditions in nitrogen. Under conditions similar to those of the present material a 20 second flushing with nitrogen before irradiation reduced the extrapolation number of the survival curve of Chinese hamster cells from 3.8 to 2.4 (ELKIND et coll 1968). In contrast, when these cells were irradiated in a different in vitro system the extrapolation number in nitrogen was similar to the aerobic one. Experiments with HeLa cells in vitro gave similar results in that the extrapolation number failed to change whether oxygen or nitrogen was the gas phase (NIAS et coll 1967).

In a general agreement with the present results no repair of the radiation damage was noted when ascaris eggs were treated with fractionated radiation doses under hypoxic conditions (LIECHTI 1929). A decreased split dose ratio was calculated also by HALL & CAVANAGH (1967) in experiments with *Vicia faba* when irradiation was applied in nitrogen instead of aerobic conditions. In contrast in split dose experiments with Chinese hamster cells exposed in nitrogen recovery was demonstrated by ELKIND et coll (1965). When cellular survival curves were determined in experiments in vivo extrapolation numbers dependent (BELL et coll 1967, PHILLIPS 1968, VAN PUTTEN & KALLMAN 1968) or independent (HEWITT & WILSON 1961, REINHOLD 1966) of the presence of oxygen during the irradiation were reported.

A common interpretation of the contradictory results is difficult at present. Apart from differences in the cellular material and experimental conditions the statistical analysis of the significance of the data is often incomplete and uncertainty often concerns also the degree of hypoxia. The interpretation of split dose experiments in vivo is in addition still complicated by the possible re-population and re-oxygenation of the irradiated tissue during the time interval between the treatments. While recovery of hypoxic cells in vivo was reported by many investigators (BERRY et coll 1967, FOWLER et coll 1965, HORNSEY 1967) recent carefully conducted experiments also indicated that the recovery of such cell may be substantially decreased (ELKIND et coll 1968, BELL et coll 1967) or absent (PHILLIPS & HANKS 1968). In a recent paper SUIT & URAVO (1969) reported that acutely hypoxic mammary carcinoma cells repaired damage rapidly and extensively. In contrast chronically hypoxic cells were less able to repair radiation injury.

The danger of hypoxic cells Considering the varying oxygen gradients prevailing in human tumors (THOMLINSON & GRAY 1955) and the finding (LITTBRAND & REVEZ 1958) that cells can survive for a prolonged period of time even when

less than $0.004 \mu\text{M}$ O_2 is available in the medium, the present results and conclusions may carry some radiotherapeutic implications. While well oxygenated portions of a tumor and normal tissues with unimpaired blood supply will suffer a larger initial radiation damage than the anoxic tumor cells, theoxic cells can probably recover but the damage to anoxic cells may not be repaired. Fractionated irradiation may therefore result in a greater additivity of the injury in the anoxic cells than in oxygenated cells. Fractionation of the radiation dose can be expected to reduce the anoxic protection ratio which, with approximately small doses, may have a value even less than 1. The potential danger of surviving therapeutic radiation would seem to be greatest with the hypoxic rather than anoxic cells since the former will not only suffer less injury but also have the capacity of repairing a part of the damage (LITTBRAND & REVESZ 1969). Transfer of cells from the initially anoxic to the hypoxic compartment as a result of partial re-oxygenation may constitute a particular hazard with regard to the success of therapy.

Conclusions

Cultures of Chinese hamster cells in Ehrlich ascites tumor and three of its substrains were exposed to roentgen radiation in oxygen or argon with an oxygen content less than 2 ppm. Significant differences in radiosensitivity between the ascites tumor and the substrains were demonstrated when irradiation was administered in argon. In oxygen the radiosensitivities were similar implying differences in the oxygen enhancement ratio between different cell strains. The survival curves after anoxic irradiation with either single or split exposure doses were exponential with extrapolation numbers to a zero dose close to unity. In oxygen the survival curves were sigmoid and the extrapolation numbers around 2. This difference between the extrapolation numbers implies a crossing of the survival curves corresponding to a dose range where theoretically no oxygen enhancement of sensitivity can be expected to occur. Experiments performed with single exposures to 125 R or less and with three exposures to 100 R, proved the correctness of this expectation and indicated that survival is independent of the presence or absence of oxygen in this dose range.

The split dose ratios calculated from the survival after single exposure and two exposures to the same dose separated by an interval of 4, 12 or 18 hours agreed well with the extrapolation numbers and were around 2 and close to 1 when irradiations were given in the presence and absence of oxygen, respectively.

Differences in the cellular concentration of non protein bound sulphhydryl groups and the ploidy grade were considered to explain the differences in the anoxic radiosensitivity between the Ehrlich ascites tumor cells and its substrains. The role of oxygen in the expression of radiation damage was considered as a

dual one acting as an essential modifying agent to both the process of sublethal recovery and lethal damage

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SUMMARY

The survival parameters of different cell lines were studied after exposure to roentgen radiation. Oxygen affected both production and repair of radiation damage. The extent of these processes varied in the different cell lines.

ZUSAMMENFASSUNG

Die Überlebensparameter verschiedener Säugetier Zell Linien wurden nach Röntgenbestrahlung untersucht. Sauerstoff beeinflusste sowohl die Bildung als auch die Reparatur des Strahlenschadens. Das Ausmass dieser Prozesse variierte in verschiedenen Zell Linien.

RÉSUMÉ

L'auteur a étudié les paramètres de survie de différentes lignées cellulaires de mammifères après exposition aux rayons de roentgen. L'oxygène a une influence aussi bien sur l'apparition que sur la réparation des radio lésions. L'importance de ces processus est variable dans les différentes lignées cellulaires.

REFERENCES

- ALPER T. Lethal mutations and cell death. *Phys in Med Biol* 8 (1963) 365
- BERENDSEN G. W. Dose survival curves of human cells in tissue culture irradiated with alpha, beta, 70 kV X and 200 kV X radiation. *Nature* 193 (1962) 1153
- , WALTER H. M. D., FOWLER J. F. and BEWLEY D. N. Effects of different ionizing radiations on human cells in tissue culture. III. Experiments with cyclotron accelerated alpha particles and deuterons. *Radiat Res* 18 (1963) 106
- BELLI J. A., DICUS G. J. and BOWTE F. J. Radiation response of mammalian tumor cells. I. Repair of sublethal damage in vivo. *J. nat. Cancer Inst.* 38 (1967) 673
- BERRY R. J. Quantitative studies of relationships between tumor cell ploidy and dose response to ionizing radiation in vivo. Modification of radiation response in a previously irradiated tumor. *Radiat Res* 18 (1963) 236
- , SCHWARZ G., ELLIS R. E. et coll. The effect of hypoxia on the skin response of mice to divided doses of 15 MeV electrons. *Int. J. Radiat. Biol.* 12 (1967) 293
- CAMISIAL R. and COSTA F. Long term in vitro cultivation of some mouse ascites tumors. Ethical ascites carcinoma. *J. nat. Cancer Inst.* 26 (1961) 172
- CASPERSON O. and RÉVÉZ L. Cytochemical measurements of protein sulphhydryls in cell lines of different radiosensitivity. *Nature* 199 (1963) 153
- CHAPMAN J. D., STURROCK J., BOAG J. W. and CROKALL J. O. Anoxia in radiobiology. *Brit. J. Radiol.* 41 (1968) 951
- DEERING R. A. and RICE JR R. Heavy ion irradiation of HeLa cells. *Radiat. Res.* 17 (1962) 774

- DEWEY D L The X ray sensitivity of *Serratia marcescens* Radiat Res 19 (1963) 64
- DITTRICH W Treffermischkurven / Naturforsch 15b (1960) 261
- EAGLE H Amino acid metabolism in mammalian cell cultures Science 130 (1959) 432
- FLKIND M M and SUTTON H Radiation response of mammalian cells grown in culture
- I Repair of X ray damage in surviving Chinese hamster cells Radiat Res 13 (1960) 556
- and WHITMORE G F The radiobiology of cultured mammalian cells Gordon and Breach New York London Paris 1967
- WHITERS H R and BELL J A Intracellular repair and the oxygen effect in radiobiology and radio therapy In Frontiers in radiation therapy and oncology Volume 3 p 55 Edited by J M Vaeth Karger Basel 1968
- SUTTON GILBERT H MOSES W B et coll Radiation response of mammalian cells grown in culture V Temperature dependence of the repair of X ray damage in surviving cells (aerobic and hypoxic) Radiat Res 25 (1965) 359
- FOWLER J F KRAFT K ELLIS R E et coll The effect of divided doses of 15 MeV electrons on the skin response of mice Int J Radiat Biol 9 (1965) 241
- GLAS U and REVEZ L Radiosensitivity of selected sub lines of a tumour cell population tested in the presence or absence of oxygen Int J Radiat Biol 6 (1963) 69
- HAIN G M Failure of Chinese hamster cells to repair sub lethal damage when X irradiated in the plateau phase of growth Nature 217 (1968) 741
- HALL F J and CAVANAGH J The oxygen effect for acute and protracted radiation exposures measured with seedlings of *Vicia faba* Brit J Radiol 40 (1967) 128
- BRADFORD J S and OLIVER R Extreme hypoxia its effect on the survival of mammalian cells irradiated at high and low dose rates Brit J Radiol 39 (1966) 302
- HARRIS R J C Cell culture and somatic variation Holt Reinhart and Winston New York 1964
- HAUSCHKA T S GRINNELL S T REVEZ L and KLEIN G Quantitative studies on the multiplication of neoplastic cells in vivo IV Influence of doubled chromosome number on growth rate and final population size J nat Cancer Inst 19 (1957) 13
- HEWITT H B and WILSON C W Survival curves for tumor cells irradiated in vivo Ann NY Acad Sci 95 (1961) 818
- HORNSEY S The recovery process in organized tissue In Radiation Research Proc 3rd Intern Congress Radiation Research Cortina 1966 p 538 Edited by G Silini North Holland Publishing Company Amsterdam 1967
- HOWARD FLANDERS P Effect of oxygen on the radiosensitivity of bacteriophage in the presence of sulphhydryl compounds Nature 186 (1960) 485
- and ALPER T The sensitivity to irradiation under controlled gas conditions Radiat Res 7 (1957) 518
- HUMPHRY R M DEWEY W C and CORK A Effect of oxygen in mammalian cells sensitized to radiation by incorporation of 5 bromodeoxyuridine into DNA Nature 198 (1963) 268
- LIECHTI A Über den Zeitfaktor der biologischen Strahlenwirkung Strahlentherapie 33 (1929) 1
- LITTEBRAND B and REVEZ L Recovery from X ray injury and the effect of oxygen Nature 203 (1964) 889
- — Survival of cells in anoxia Brit J Radiol 41 (1968) 479
- — The effect of oxygen on cellular survival and recovery after radiation Brit J Radiol 42 (1969) 914

- — Radiation damage and repair in cystamine treated cells To be published in *Acta radiol Ther Phys Biol*
- NAS A H W GREENE D FOX M and THOMAS R L Effect of 14 MeV monoenergetic neutrons on HeLa and P388F cells in vitro *Int J Radiat Biol* 13 (1967) 449
- NIELSEN K Chromosome studies in the Ehrlich ascites tumor of the mouse grown in vitro *Hereditas* (Lund) 58 (1967) 73
- PILLIPS T L Qualitative alteration in radiation injury under hypoxic conditions *Radiology* 91 (1968) 529
- and HANAS G E Apparent absence of recovery in endogenous colony forming cells after irradiation under hypoxic conditions *Radiat Res* 33 (1968) 517
- PORTER H Extrapolation numbers *Brit J Radiol* 36 (1963) 372
- POWERS W E and TOLMACH L J A multicomponent X ray survival curve for mouse lymphosarcoma cells irradiated in vivo *Nature* 197 (1963) 710
- REINHOLD H Quantitative evaluation of the radiosensitivity of cells of a transplantable rhabdomyosarcoma in the rat *Europ J Cancer* 2 (1966) 33
- RÉVÉZ L and LITTBAND B Variation of the relative sensitivity of closely related neoplastic cell lines irradiated in culture in the presence or absence of oxygen *Nature* 203 (1964) 747
- — (a) Fractionated irradiation and the effect of oxygen *In* Progress in Radiology *Excerpta Medica Intern Congress Series No 105 p 1476 Amsterdam 1967*
- — (b) Qualitative differences between cellular radiation damage suffered underoxic and anoxic conditions the dual effect of oxygen *Proc Intern Conference on Radiation Biology and Cancer Kyoto 1966 p 67 Radiation Society of Japan 1967*
- — The dual effect of oxygen *Studia Biophysica* 15/16 (1969) 197
- — The composite radioprotective and radiosensitizing effect of an organic free radical *In* Radiation protection and sensitization Taylor & Francis London 1970
- BERGSTRAND H and MODIG H (a) Intrinsic non protein sulphhydryl levels and cellular radiosensitivity *Nature* 198 (1963) 175
- GLAS U and HILDING G (b) Relationship between chromosome number and radiosensitivity of tumour cells *Nature* 198 (1963) 260
- LITTBAND B and MODIG H Variation in the response of different cells within a tumour to treatment with X rays or alkylating substances *In* Control of cellular growth in the adult organisms p 374 Edited by H Teir and T Rytoma Academic Press London 1967
- ROBINSON J E and RÉVÉZ L Sensitivity of mammalian cells in vitro to X irradiation in air and in absence of oxygen *Radiat Res* 14 (1961) 493
- SHALEK R J and SMITH C E The radiolysis of dilute solutions of lysozyme I Enzymatic studies relating to the oxygen effect *Int J Radiat Biol* 14 (1968) 9
- SILVER G and HORNSLEY S Studies on cell survival of irradiated Ehrlich ascites tumour III A comparison of the X ray survival curves obtained with a diploid and a tetraploid strain *Int J Radiat Biol* 5 (1967) 147
- SURR H and URANO M Repair of sublethal radiation injury in hypoxic cells of a C₃H mouse mammary carcinoma *Radiat Res* 37 (1969) 423
- THOMLINSON H H and GRAY L H The histological structure of some human lung cancers and the possible implications for radiotherapy *Brit J Cancer* 9 (1955) 539
- VAN PUTTEN L M and KALLMAN R F Oxygenation status of a transplantable tumor during fractionated radiation therapy *J nat Cancer Inst* 40 (1968) 441

CONTROLE DOSIMETRIQUE EN BRACHYCURIETHERAPIE PAR LES ISODOSES 'TSCARGOT'

par

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Nous présentons une modification pratique du calcul de la dose en brachycurietherapie par iridium 192. Il s'agit d'une adaptation de notre technique sur points, systematisée en 1960 (PIERQUIN et coll 1960 et 1962) et modifiée une première fois en 1964 par l'emploi des isodoses circulaires standard (PIERQUIN et coll 1964).

Rappel des isodoses circulaires standard. Ce controle dosimetrique s'associe à l'étude previsionnelle (PIERQUIN et coll 1968) établie pour chaque malade. Son but est de calculer après la mise en place du dispositif radifere la repartition de la dose en différents points à l'intérieur et autour de ce dispositif forme de lignes radio actives continues et paralleles. Cette dosimetrie est établie dans un ou plusieurs plans perpendiculaires aux lignes radio actives. Ce ou ces plans de coupe sont obtenus par tomographie ou par reconstruction. Les images des fils ou aiguilles apparaissent sur la tomographie ou le schema de reconstruction sous forme de points. Ces points et les contours des structures anatomiques reconnues sont dessinés sur un calque.

Des isodoses circulaires standard gravees sur des tampons encreurs en caoutchouc ont imprimees sur le calque autour de chaque point. Elles ont ete calculees a partir d'un facteur k arbitraire de valeur 1 R/h 1 mCi/cm pour des doses de 10—4—2—1—0.6—0.4—0.2 R. Un jeu de dix tampons permet de disposer d'isodoses standard pour des longueurs radio-actives de 1—2—3—4—5—6—7—8—10—12 cm.

Par recouplement entre ces differentes isodoses circulaires la dose peut etre calculee en n'importe quel point du plan de coupe. En quelques minutes on peut ainsi calculer la dose de base et la dose de reference, et finalement dessiner toutes les isodoses desirables.

Imperfections des isodoses circulaires standard Cette methode de calcul de la dose comporte quelques imperfections enumerees ci-dessous.

- 1 Les isodoses sont calculees en roentgens et non en rads
- 2 Leur universalite ne permet pas d'introduire le coefficient d'attenuation dans les tissus coefficient variable d'un isotope a un autre
- 3 La lecture des isodoses devient difficile lorsque le dispositif radifere est trop serre et trop nombreux des fautes d'identification deviennent possibles
- 4 Inversement les espacements entre les isodoses d'un meme tampon sont relativement importants d'ou des risques d'erreur d'interpolation dans le calcul de la dose en tel ou tel point du volume cible (surtout dans l'espace compris entre l'isodose 10 et l'isodose 4)
- 5 Un leger deplacement des isodoses (de l'ordre du millimetre) par rapport au point de section de la ligne radio-active est pratiquement inevitable au moment du tamponnement.

Toutes ces imperfections peuvent aboutir a des erreurs de calcul de l'ordre de 10 %. Elles se compensent bien souvent mais elles laissent une marge d'incertitude non negligeable.

Les isodoses 'escargot'

A l'encontre des isodoses tampons centrees sur les differents points de section des lignes radio-actives ces isodoses escargot sont placees sur chaque point du moral etudie pour la recherche de la dose de base de la dose de reference ou d'une autre dose. Elles n'interessent qu'un seul radio-element en l'occurrence l'Iridium 192 ce qui permet d'introduire dans le calcul le coefficient d'attenuation et la valeur de la constante radio-active (facteur k). Elles ont ete dessinees a partir d'un calcul sur ordinateur a l'aide d'un traceur de courbes.

Pratiquement il s'agit d'un jeu d'isodoses inscrites sur trois feuilles transparentes en rhodoid. La premiere feuille contient les isodoses pour des longueurs

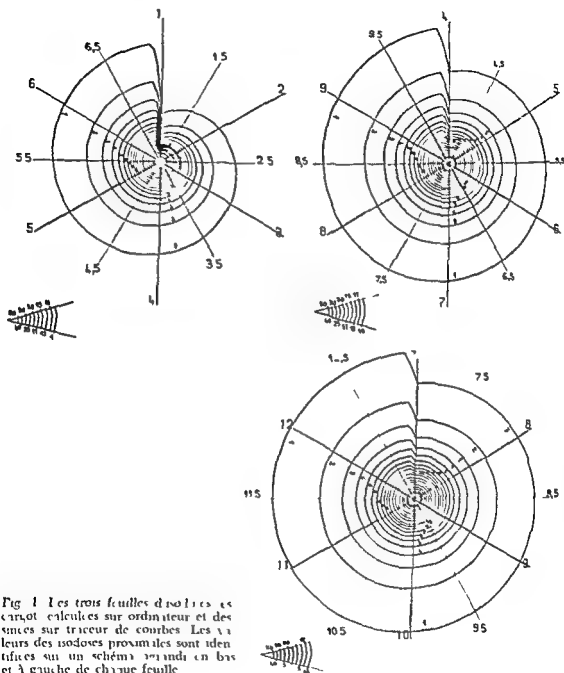


Fig. 1. Les trois feuilles d'isolignes et caractéristiques calculées sur ordinateur et dessinées sur traceur de courbes. Les valeurs des isodoses proximales sont identiques au schéma joint en bas et à gauche de chaque feuille.

radioactives comprises entre 1 et 6,5 cm. La seconde feuille va de 1 à 9,5 cm et la troisième feuille de 7 à 12,5 cm. Les lignes radioactives sont donc étudiées de 0,5 cm à 0,5 cm. Elles se disposent en rayons de roue autour du point central. De cette façon, en choisissant une seule de ces feuilles par leur chevauchement, on peut couvrir dans la plupart des cas toutes les longueurs utilisées dans le



Fig 2 Six fils d'iridium 192 de 3 cm de longueur implantés dans un carcinome épidermoïde de la langue mobile Tomographie transversale de contrôle Agrandissement 1.33

dispositif radifère considère que ces longueurs soient égales à un nombre entier de centimètres ou non

Ces différentes isodoses sont au nombre de 19 à savoir 50—40—30—25—20—17—15—13—11—10—9—8—7—6—5—4—3—2—1 rad/heure pour une activité de 1 mCi/cm (iridium 192). L'inscription continue pour chaque valeur et entre les différentes longueurs radio-actives détermine des isodoses de forme hélicoïdale d'où le sobriquet d'isodoses escargot (Fig 1). Ces isodoses sont bien entendu agrandies d'un facteur 1.33 qui correspond au coefficient de grandissement des tomographies.

Le calcul s'effectue de la façon suivante:

Le calque de la tomographie ou de la reconstruction agrandie du facteur 1.33 est placé sur une plaque de plexiglas perforée en son centre.

La feuille adéquate d'isodoses escargot est placée sur ce calque en solidarifiant son point central avec le point tumoral étudié à l'aide d'une épingle simple perforant simultanément la feuille d'isodoses, le calque et la plaque de plexiglas.

En faisant tourner la feuille d'isodoses autour de son point central on met successivement en coïncidence le point de section de chaque ligne radio-active apparaissant sur le schéma avec le rayon de la feuille d'isodoses qui correspond

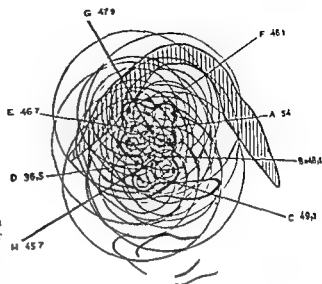


Fig 3 Dosimétrie de l'implantation (voir la figure 2) par la technique sur points avec tampons en isodoses circulaires standard. Isodose de référence 40 R/heure pour 1 mCi/cm

sa longueur. En quelques dizaines de secondes on peut ainsi totaliser la dose recue de l'ensemble du dispositif radifère au niveau du point tumoral ou peritumoral étudié.

Nous conseillons de calculer tout d'abord la dose au niveau du ou des différents points de la dose de base puis de dessiner la limite du volume cible soumise, enfin de calculer quelques points tests sur cette limite afin de déterminer la position définitive de l'isodose de référence au niveau de laquelle sera calculée la dose tumorale totale. Avec un peu d'expérience, ce contrôle dosimétrique peut être réalisé en quelques minutes (Figs 2—4).

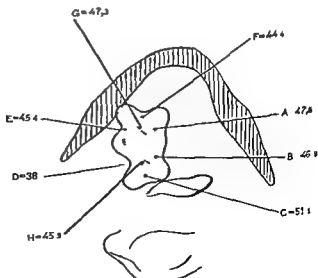


Fig 4 Dosimétrie de la même implantation (voir les figures 2 et 3) par la technique sur points avec des isodoses escargot. Isodose de référence 40 rad/heure pour 1 mCi/cm. Dans ce cas considéré, la dose calculée en R (figure 2) aurait conduit pour un même volume cible apparent à une surévaluation par rapport à la dose calculée en rad (figure 3).

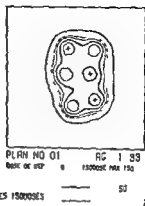


Fig 5 Dosimetrie de la meme implantation (voir les figures 2-4) par ordinateur a et traceur de courbes. L'isodose de reference 40 rad/heure pour 1 mCi/cm est superposable à celle dessinée à partir des isodoses 'escargot'.

Discussion

Ces isodoses 'escargot' comportent plusieurs avantages

- 1 La précision du dessin grâce au calcul de ces isodoses par ordinateur leur inscription directe par traceur de courbes
- 2 Le grand nombre de valeurs disponibles tant en longueurs radio-actives qu'en isodoses : les interpolations sont de ce fait réduites à des intervalles négligeables
- 3 La large surface de ces feuilles d'isodoses permettant d'inclure des isodoses de faible valeur à grande distance du point étudié. Ces isodoses 'escargot' sont donc utilisables pour de grands volumes-cibles
- 4 La clarté de la lecture malgré le grand nombre d'isodoses le calcul comportant que les marques des points de section des lignes radio-actives et des points tumoraux ou péri-tumoraux étudiés
- 5 Le faible investissement de ce matériel et son absence d'encombrement : dix tampons de l'ancien système se trouvant remplacés par trois feuilles de papier

Quelques réserves sont cependant à formuler

- 1 Le resserrement des isodoses de valeur élevée implique une lecture attentive pour éviter des erreurs d'interpolation
- 2 La délimitation de l'isodose de référence (ou de toute autre isodose à l'intérieur ou autour du volume cible) n'est plus guidée par le recoupement des isodoses circulaires des tampons : il faut apprendre à dessiner l'isodose de référence dans le vide à partir de quelques points judicieusement calculés sur la limite de ce que l'on prévoyait être dans l'enveloppe du volume traité
- 3 Le facteur d'agrandissement 1,33 nécessite de corriger les mesures directes en cas de reconstruction

tomographe (Il reste toujours possible de faire dessiner les isodoses escargot sans agrandissement, mais la lecture sur le calque devient alors très difficile, les isodoses étant trop rapprochées. L'agrandissement est en fait une nécessité pour obtenir une lecture claire.)

4 Ces isodoses escargot n'ont été calculées que pour l'iridium 192. Cette limitation, indispensable pour la précision de la dosimétrie, ne peut plus être maintenant considérée comme un handicap du fait de la généralisation de l'emploi de l'iridium 192 en brachycurietherapie avec lignes radioactives parallèles (bien entendu, il est loisible de calculer, selon la même méthodologie, des isodoses escargot pour d'autres radioéléments).

RÉSUMÉ

Les isodoses escargot permettent de simplifier le contrôle dosimétrique en brachycurietherapie par iridium 192 avec lignes radioactives parallèles. Grâce au calcul sur ordinateur et au dessin sur traceur de courbes, elles permettent d'effectuer une dosimétrie avec précision accrue. Leur prix de revient et leur encombrement négligeables permettent de les utiliser dans tous les centres de radiotherapie disposant d'iridium 192.

SUMMARY

The dosimetric control in brachycurietherapie with iridium 192 and parallel radioactive lines can be simplified determining escargot isodoses. Dosimetry can in this way be carried out with great precision by means of computer calculation and tracing of curves. The costs and space requirements are negligible and the method can be used in all radiotherapeutic centres where iridium 192 is employed.

ZUSAMMENFASSUNG

Escargot Isodosen erlauben eine Vereinfachung der Kontrolle der Dosimetrie bei der Brachycurietherapie mit Iridium 192 und radioaktiven parallelen Linien. Es ist möglich die Dosimetrie mit verbesserter Genauigkeit durch Berechnungen mit einem Computer und Aufzeichnen von Kurven zu plannieren. Die Kosten und der Platzaufwand sind zu vernachlässigen und die Anlage kann von allen radiotherapeutischen Zentren die über Iridium 192 verfügen verwendet werden.

BIBLIOGRAPHIE

- DUTREIX A. Utilisation d'un ordinateur pour la dosimétrie en curietherapie. *Ann. Physiol. Biol. méd.* 2 (1967) 139.
- PIERQUIN B. et CHASSAGNE D. Isodoses circulaires standard en endo- et plésiocurietherapie. *Acta radiol. Ther. Phys. Biol.* 2 (1964) 449.
- and FAYOS J. V. Dosimetry by tomography in interstitial curietherapie: point technique. *Amer. J. Roentgenol.* 87 (1962) 585.
- CHASSAGNE D. et GASIOROWSKI M. Technique de dosimétrie en curietherapie interstitielle par tomographie transversale. *Acta radiol.* 53 (1960) 314.
- DUTREIX A., PAINE C. et coll. Endocurietherapie. A dosimetric study of two parallel radioactive sources. *Acta radiol. Ther. Phys. Biol.* 7 (1968) 193.

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BIBLIOGRAPHIE

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- PIERQUIN B. et CHASSAGNE D. Isodoses circulaires standard en endo- et plésiocurietherapie. *Acta radiol. Ther. Phys. Biol.* 2 (1964) 449.
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Table 2

Life expectation in malignant lymphogranulomatosis — Influence of histologic type on survival time according to LUKES & BUTLER (1966) — Freiburg patient data 1948—1967

Histologic type	Numbers	Survival rates in per cent		
		3 year	5 year	10-year
Lymphocytic and histiocytic nodular	11	72.8	46.9	46.2
Lymphocytic and histiocytic diffuse	13	76.9	68.6	58.2
Nodular sclerosis	88	74.5	56.1	36.4
Mixed	40	49.8	36.6	30.5
Diffuse fibrosis	21	43.5	43.5	11.6
Reticular	20	48.1	30.1	30.1
Total	193	63.8	49.0	33.7

involvement on one side of the diaphragm without and with localized organ extension)

The cured patients belong to both clinical forms without as well as with symptoms or signs of active disease: the number of patients without signs is only slightly higher than those with signs (Table 1)

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The histologic appearances of the lymphocytic and histiocytic forms consist of a distinct predominance of lymphocytes and histiocytes and only a few Hodgkin and Sternberg giant cells. According to LUKES et coll. (ref. 10), as well as to the similar Freiburg classification these forms carry the best prognosis (Table 2)

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Table 3 indicates that most of the twenty classifiable patients considered as potentially cured belonged to the nodular sclerosis group: approximately 40%

Table 1

Distribution of 28 cured patients (13 males and 15 females) according to stage and clinical form of Hodgkin's disease

Clinical form	Stages according to the Rye classification				Number of patients
	I	II	III	IV	
A	6	10	—	—	16
B	3	5	—	4	12
Total	9	15	—	4	28

were evaluated. A classification by the LUKES method was made in four of six patients after the detailed report of histologic investigation, while in a further six patients the diagnosis had been cytologically verified.

Results

The treatment of the 393 patients has been discussed in detail elsewhere (MUSSHOFF & BOUTIS ref. 12, 13, 14). The 5 year survival rate of this series amounted to 48.6 %, the 10 year survival rate to 29.3 %, while the 15- and 20 year survival rates were each 23.1 %.

Spread, signs, histologic types and treatment of the twenty-eight cured patients are presented in Tables 1, 3 and 4. The clinical stage was evaluated according to the classification proposed at the Lymphogranulomatosis Symposium at Rye, New York, 1965, and the clinical form of the disease, without (type A) and with (type B) evidence of general involvement, was judged by the criteria of the authors (ref. 16).

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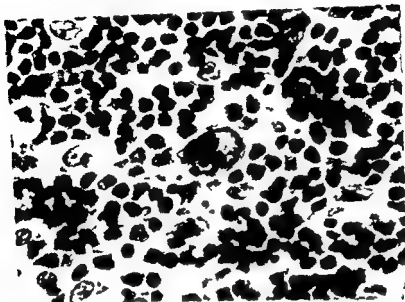


FIG. 1. Case 1 (stage I A). L+H diffuse type. Diffuse interspersed of lymph node by mature lymphocytes. Typical Sternberg giant cell with large nucleolus with translucent centre. $\times 900$.

(FREIDBERG ref. 1), amounted to 22.1% (the 5 year survival rate of the total series in the period 1948—1967 being 48.6%) (ref. 12, 13, 14). The concept of cure is thus based upon the fact that the reference curve of patients in the first remission group decreases exponentially and rapidly during the first years after treatment becomes flatter in the years that follow and reaches a constant level at the ninth year of remission (ref. 11). No recurrence was recorded in the period 1948—1967 after the ninth year and the statistical probability of a recurrence after 9 years remission is therefore small. A patient in whom full remission of 9 years or longer is achieved after the first treatment sequence may be looked upon as cured. This premise was taken as a basis for the evaluation of cure used in this investigation.

Analysis of the cured patient series as regards clinical aspects, histology and treatment produced the following results.

1. Cures were achieved only as long as the disease had not crossed the diaphragm, whether from above or below. Only in one of the twenty-eight patients were the lesions localized infradiaphragmatically (stages I and II, according to Rye). The previously comparatively unfavourable prognosis for infradiaphragmatic locations in clinical stage II and especially stage III (Rye), was due to

Table 3

Distribution according to histologic type (LUKES & BUTLER) and clinical stage (Rye) in cured Hodgkin's disease

Histologic classification (LUKES & BUTLER)	Num bers	Stage and clinical form							
		I		II		III		IV	
		A	B	A	B	A	B	A	B
Lymphocytic and histiocytic nodular	—	—	—	—	—	—	—	—	—
Lymphocytic and histiocytic diffuse	4	3	—	1	—	—	—	—	—
Nodular Sclerosis	11	1	—	5	3	—	—	—	2
Mixed	4	1	—	2	—	—	—	—	1
Diffuse fibrosis	—	—	—	—	—	—	—	—	—
Reticular	1	1	—	—	—	—	—	—	—
Total patients	20	6	—	8	3	—	—	—	3

(207) of all patients examined in the period 1948—1967 (508) belonged to this nodular sclerosis group. The lymphohistiocytic diffuse form, i.e. the lymphocyte predominant form and the mixed form were present in four of the cured patients. A rare reticular form, previously characterized as Hodgkin sarcoma (JACKSON & PARKER, ref. 3), was present in one patient.

Therapy in cured malignant lymphogranulomatosis The involved areas were treated with roentgen rays at 180 to 250 kV, adjacent regions were excluded. The radiation dosage in the patient group with lymphocytic or histiocytic proliferation was 2 700 to 3 800 rad, in the group with nodular sclerosis 1 500 to 4 000 rad, in the mixed cell type exposure dose 2 750 R to 4 230 rad, and in the reticular type exposure dose 2 900 R (Table 4). Among the twenty patients classified according to LUKES' scheme, fifteen received chemotherapeutic treatment with nitrogen mustard or cyclophosphamide concurrently or alternately. Of the total of twenty-eight cured patients, twenty-three were given additional chemotherapeutic treatment. The ratio of the patients treated by combined radiation and chemotherapy and those by radiation therapy alone therefore becomes 3 (46) : 1 in the group of the 20 (28) cured patients, and 6 : 1 in the total of 193 patients treated during the period. None of the cured patients was treated by chemotherapy alone.

Discussion

A certain percentage of patients with Hodgkin's disease can be cured. The percentage during the period 1948—1958, calculated by the life table method

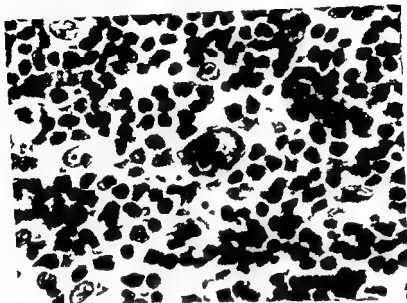


Fig 1 Case 1 (stage I A) L+H diffuse type Diffuse interspersion of lymph node by mature lymphocytes Typical Sternberg giant cell with large nucleolus with translucent centre $\times 900$

(FREUDENBERG ref 1) amounted to 22.1% (the 5 year survival rate of the total series in the period 1948—1967 being 48.8%) (ref 12 13 14). The concept of cure is thus based upon the fact that the reference curve of patients in the first remission group decreases exponentially and rapidly during the first years after treatment becomes flatter in the years that follow and reaches a constant level at the ninth year of remission (ref 11). No recurrence was recorded in the period 1948—1967 after the ninth year and the statistical probability of a recurrence after 9 years remission is therefore small. A patient in whom full remission of 9 years or longer is achieved after the first treatment sequence may be looked upon as cured. This premise was taken as a basis for the evaluation of 'cure' used in this investigation.

Analysis of the cured patient series as regards clinical aspects, histology and treatment produced the following results:

1. Cures were achieved only as long as the disease had not crossed the diaphragm, whether from above or below. Only in one of the twenty-eight patients were the lesions localized infradiaphragmatically (stages I and II according to Rye). The previously comparatively unfavourable prognosis for infradiaphragmatic locations in clinical stage II and especially stage III (Rye) was due to

Table 4

Treatment in 20 of 28 cured patients in whom classification according to LUKES & BUTLER was possible

Case & sex	Stage	Spread of the disease
<i>Lymphocytic and histiocytic diffuse type (see Fig. 1)</i>		
1 ♂	I A	Right side of neck
2 ♂	I A	Left side of neck and supraclavicular fossa
3 ♂	I A	Left supraclavicular fossa
10 ♀	II A	Left side of neck and supraclavicular fossa Right side of neck and supraclavicular fossa Mediastinum
<i>Nodular sclerosis (see Fig. 2)</i>		
4 ♂	I A	Left side of neck and supraclavicular fossa
11 ♀	II A	Left side of neck and supraclavicular fossa Mediastinum
12 ♀	II A	Left supraclavicular fossa Right axilla and infraclavicular fossa
13 ♂	II A	Left side of neck Left supraclavicular fossa Mediastinum
14 ♀	II A	Right side of neck supraclavicular fossa and right axilla Left side of neck supraclavicular fossa and left axilla
15 ♂	II A	Right supraclavicular fossa Mediastinum
20 ♂	II B	Both groins
21 ♀	II B	Mediastinum Right supraclavicular fossa
22 ♀	II B	Right side of neck Right supraclavicular fossa Mediastinum Left supraclavicular fossa Left infraclavicular fossa
25 ♀	IV B	Mediastinum sternum and right lung Left axilla Right axilla Right supraclavicular fossa Left supraclavicular fossa
26 ♂	IV B	Thoracic spine Left side of neck and supraclavicular fossa
<i>Mixed type (see Fig. 3)</i>		
5 ♂	I A	Left side of neck supraclavicular and infraclavicular fossae
16 ♀	II A	Right supraclavicular fossa Left supraclavicular fossa Mediastinum
17 ♀	II A	Left supraclavicular fossa Right supraclavicular fossa Mediastinum
27 ♂	IV B	Mediastinum and right paramediastinal pulmonary regions
<i>Reticular type (see Fig. 4)</i>		
18 ♂	I A	Left axilla

Table 4 (cont.)

Rad at on treatment	Chemotherapy	Remission
3 000 rad/26 days	—	> 13 years
2 800 R/17 days	—	> 13 years
3 000 R/18 days	—	> 11 years
3 500 rad/25 days	N mustard 44 mg/18 days	> 12 years
2 700 rad/20 days	Cortisone 2.6 g/51 days	> 12 years
3 800 rad/26 days		
4 000 rad/26 days	N mustard 26.5 mg/11 days	> 15 years
2 600 R/11 days	M mustard 47 mg/20 days	> 13 years
1 500 rad/11 days	Cortisone 1.650 g/33 days	
4 000 rad/26 days	Sanamycin unknown dose	
3 600 rad/21 days	N mustard 31 mg/50 days	> 12 years
3 100 rad/29 days	Decortin 1.32 mg/68 d ys	
3 800 rad/37 days	N mustard 26.5 mg/34 days	> 13 years
3 750 rad/30 days	Decortin 500 mg/36 days	
3 000 R/17 days	Cyclophosphamide 7.1 g/39 days	> 9 years
3 900 rad/34 days	Decortin 1.16 g/56 days	
3 200 R/23 days	N mustard 36 mg/37 days	> 9 years
3 300 rad/23 day	Decortin 870 mg/40 days	
2 500 R/23 days	N mustard 41 mg/20 days	> 13 years
3 600 ad/37 days	Sanamycin 5.900 gamma/19 days	
3 300 R/19 days	N mustard 34.5 mg/20 days	> 12 years
2 800 rad 25 days	Cortisone 5.125 g/61 days	
3 300 rad/41 days	Cyclophosphamide 2.9 g/14 days	> 9 years
3 200 ad/25 days	Decortin 875 mg/53 days	
2 800 R/17 days		
1 800 R/7 days		
4 000 rad/22 days	N mustard 25 mg/10 days	> 14 years
2 600 R/14 days		
2 700 R/19 days		
2 800 R/15 days		
2 500 R/10 days		
3 400 rad 20 days	Diclofen 39 mg/59 days	> 9 years
3 000 rad/26 days	Decortin 930 mg/64 days	
3 000 R/16 days	N mustard 14 mg/6 days	> 14 years
2 700 R/33 days	Sanamycin 3.400 gamma/25 days	> 11 years
2 500 R/28 days	Cortisone 1.650 mg/17 days	
4 230 r d 31 days		
2 400 R/28 days	—	> 11 years
2 400 R/30 days		
2 900 rad 25 days		
3 500 rad 38 days	N mustard 36 mg/20 days	> 19 years
2 900 R/36 days	Decortin 280 mg/22 days	

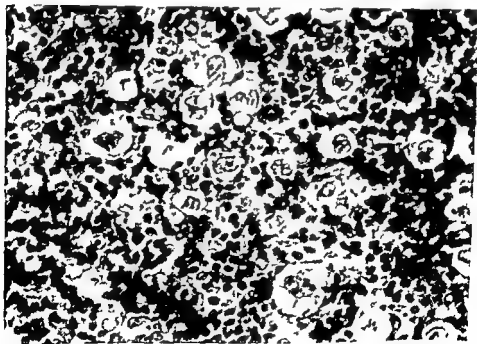


Fig 2 Case 26 (stage IV B) Nodular sclerosis type *Upper view* Knotty broad collagenous fibre bands in centre *v* Gieson *Lower view* Lymphocytes atypical reticular cells and Sternberg giant cells $\times 360$

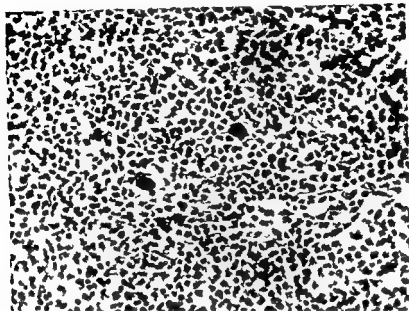


FIG. 3 Case 5 (stage I A) Mixed type Varying cell composition with Hodgkin cells Sternberg giant cells histiocytes eosinophil leukocytes plasma cells and slight fibrosis $\times 360$

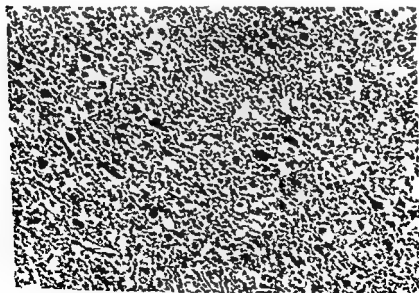


FIG. 4 Case 6 (stage I A) Reticular type Predominately atypical reticular cell Sternberg giant cells and Hodgkin cells slight fibrosis and on the com-

the fact that early diagnosis and thus timely treatment was not possible in the 1940s and 1950s before the introduction of diagnostic lymphography.

One patient in the series has been classed in stage III (R_{yc}) with supra- and infradiaphragmatic lymph node involvement and in the eighth year of his first remission, he therefore almost fulfills the statistical qualifications for complete healing. VERA PETERS and KAPLAN (personal communications) have also had several cured patients in stage III.

Systematic irradiation of all supra- and infradiaphragmatic lymph node sites in stage III with telecobalt was used from 1964 (ref. 27) for twenty of the twenty-five patients in stage III. (One patient died before infradiaphragmatic irradiation could be commenced after the supradiaphragmatic irradiation, and two patients discharged themselves before termination of therapy. Endolymphatic therapy took the place of infradiaphragmatic percutaneous irradiation in two patients.) The remission rate in the series treated in this way thus amounted to 81%. It would therefore appear that patients with both supra- and infradiaphragmatic lymph node involvement stage III (R_{yc}) may now be curable.

2 The presence of clinical symptoms and signs of the disease (clinical form B) does not exclude cure. The ratio of cured patients with active manifestations to those without in the present series is 4 to 3. This is contrary to the widely held opinion that manifestations according to clinical form B should be regarded as a contraindication to further local treatment.

3 Sex has no important influence on cure. In spite of the larger number of males in the series, the females outnumbered the males by about 3:2.

4 Cure is evidently possible in all histologic forms. This is true for the lymphocytic and histiocytic predominant groups and the lymphocyte-depleted forms, as well as for nodular sclerosis and mixed forms. By far the largest number of cured patients (three out of four) belong, however, to the prognostically favourable lymphocyte-predominant forms and the nodular sclerosis group, as was to be expected. The approximate ratio of possible cure when compared in the four histologic groups, i.e. the lymphocytic predominance, the nodular sclerosis, the mixed type and the lymphocytic depletion groups is 7:5:4:1 in the whole material.

5 All cured patients were treated by local radiation therapy (180 to 250 kV). Some of the cured patients received additional chemotherapy. One patient with reticular fibrosis in stage I was given combined surgical and radiation treatment. None of the cured patients was treated by chemotherapy alone. Present-day therapeutic possibilities suggest that complete cure is attainable — if the few cases in the literature treated purely by surgery are disregarded (SLAUGHTER ref. 25, 26) — by irradiation alone.

The doses given to cured patients varied from 1 500 to 4 000 rad. This points

to the fact that the radiation dose to be employed for destroying the malignant tissue in lymphogranulomatosis cannot be fixed as it must differ from patient to patient (PAPILLOV et coll (ref 21) made the same observation.) The level of the tissue destructive dose, empirically determined previously to 4 000 R focal dose (ref 4 19), is the one which is tissue destructive for practically all (more than 90 %) Hodgkin granulomas. As long as the specific tissue destructive dose for each patient with lymphoma malignum Hodgkin is not calculable in advance the empirically determined destructive dose of 4 000 rad should for security reasons always be given.

Acknowledgements

The authors take this opportunity of thanking Professors Bohle Bungeler and Wurm and Dr Fischer for certain histologic preparations. They are also indebted to Mr A. Low for the translation.

SUMMARY

Of a series of 393 patients with Hodgkin's disease treated during the period 1948—1967 29.1 % was cured. Twenty seven of the patients had supradiaphragmatic and only one infradiaphragmatic lymph node involvement in stages I and II (Rye) while four had extension to a local organ (stage IV). Almost the same number of males and females were affected. All patients were irradiated and some received additional chemotherapy. Lymph node extirpation was performed in one patient.

ZUSAMMENFASSUNG

Erfolgreiche Heilung konnte in 22.1 Prozent von 393 Patienten mit Hodgkinscher Erkrankung die in den Jahren 1948—1967 behandelt wurden festgestellt werden. Siebenundzwanzig Patienten hatten Lymphknotenbefall oberhalb des Zwerchfelles und nur ein Patient unterhalb des Zwerchfelles im Stadium I und II (nach Rye) und vier Patienten hatten Befall eines benachbarten Organes (Stadium IV). Weibliche und männliche Patienten waren ziemlich gleichmässig betroffen. Sämtliche Patienten erhielten Strahlenbehandlung und einige erhielten ebenfalls Chemotherapie. Exstirpation von Lymphknoten wurde in einem Patienten vorgenommen.

RÉSUMÉ

Sur 393 malades atteints de maladie de Hodgkin traités au cours de la période 1948—1967, un total de 22.1 pour cent ont été guéris. Vingt sept de ces malades avaient une atteinte ganglionnaire sus-diaphragmatique et un seul malade une atteinte ganglionnaire sous-diaphragmatique au stade I et II (Rye) et quatre malades avaient une extension localisée à un organe (stade IV). Les hommes et les femmes étaient atteints presque dans les mêmes proportions. Tous les malades ont été irradiés et certains ont subi en plus une chimiothérapie. L'exstirpation des ganglions a été faite chez un malade.

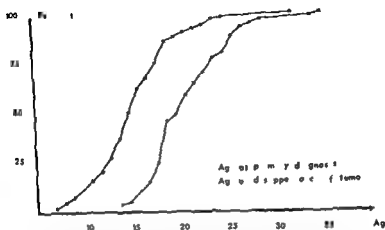
REFERENCES

- 1 FREUDENBERG K. Grundriss der medizinischen Statistik. Schattauer Verlag Stuttgart 1962
- 2 HAMANN W, OEHLERT W, MUSSHOF K and NUSS A. Histologic classification of Hodgkin's disease and its prognostic validity
- 3 JACKSON JR H and PARKER JR F. Hodgkin's disease and allied disorders. Oxford University Press New York 1947
- 4 KAPLAN H S. Role of intensive radiotherapy in the management of Hodgkin's disease. Cancer 19 (1966) 356
- 5 — Radiothérapie de la maladie de Hodgkin à la Stanford University School of Medicine. Résultats lointains. Nouv. Rev. franç. Hémat. 4 (1966) 73
- 6 — Technique statistique d'analyse des résultats de la radiothérapie de la maladie de Hodgkin. Nouv. Rev. franç. Hémat. 11 (1966) 115
- 7 — Evidence for a tumoricidal dose level in the radiotherapy of Hodgkin's disease. Cancer Res. 26 (1966), 1221
- 8 — Long term results of palliative and radical radiotherapy of Hodgkin's disease. Cancer Res. 26 (1966) 1250
- 9 LUKES R J and BUTLER J J. The pathology and nomenclature of Hodgkin's disease. Cancer Res. 26 (1966) 1063
- 10 — — and HICKS E B. Natural history of Hodgkin's disease as related to its pathologic picture. Cancer 19 (1966) 317
- 11 MUSSHOF K und BOUTIS L. Die Frage der Heilbarkeit der Lymphogranulomatose beurteilt nach den Behandlungsergebnissen der Freiburger Medizinischen Klinik. Ver. dtsch. Ges. inn. Med. 73 (1967) 322
- 12 — — Therapy results of Hodgkin's disease. Freiburg J. Br. 1948—1966. Cancer 21 (1968) 110
- 13 — — Beurteilung Behandlungsgrundsätze und -ergebnisse bei der malignen Lymphogranulomatose. Strahlentherapie (1968) Sonderband 69 S. 59
- 14 — — Die Behandlungsergebnisse der malignen Lymphogranulomatose in Abhängigkeit von individuellen und krankheitsspezifischen Faktoren. Freiburger Ergebnisse 1948—1967. Klin. Wschr. 47 (1969) 93
- 15 — BUSCH K M und KAMINSKI H. Lymphogranulomatose (Morbus Hodgkin) mit Knochenbefall. Symptomatologie mit besonderer Berücksichtigung des Röntgenbildes. Therapie und Prognose. Ein Bericht über 66 Fälle (Freiburger Krankengut 1948—1961). Fortschr. Röntgenstr. 101 (1964) 117
- 16 — BOUTIS L, STRICKSTROCK K H und MERTEN D. Die Behandlung der Lymphogranulomatose. Dtsch. med. Wschr. 92 (1967) 1603
- 17 — RENEMANN K H, BOUTIS L and AFKHAM J. The differentiation of two different types of stage IV of Hodgkin's disease. Progress in lymphology II. Miami 1968
- 18 — — — Die extranoduläre Lymphogranulomatose (Hodgkin's disease). Diagnose, Therapie und Prognose bei zwei unterschiedlichen Formen des Organbefalls. Ein Beitrag zur Stadieneinteilung der Lymphogranulomatose. Fortschr. Röntgenstr. 109 (1968) 776
- 19 — STAMM H, LUMMEL G und GOSSEL K. Zur Prognose der Lymphogranulomatose. Klinisches Bild und Strahlentherapie. In: Beiträge der inneren Medizin S. 549. Herausgegeben von Keiderling. Schattauer Verlag Stuttgart 1964
- 20 OEHLERT W und HAMANN W. Zur Histologie der potentiell geheilten Lymphogranulomatose. Patienten im Freiburger Krankengut. Strahlentherapie (1968) Sonderband 69 S. 113

- 21 PAPILLON J CROIZAT P REVOL L, *in* coll Les survies de plus de 10 ans dans la maladie de Hodgkin *Nouv Rev franç Hémat* 6 (1966) 79
- 22 PETERS M V Prophylactic treatment of adjacent areas in Hodgkin's disease *Cancer Res* 26 (1966) 1232
- 23 — Discussion Long term results of radical radiotherapy of Hodgkin's disease *Cancer Res* 26 (1966) 1253
- 24 — Résultats lointains du traitement de la maladie de Hodgkin *Nouv Rev franç Hémat* II (1966) 60
- 25 SLAUGHTER D P Radical surgery *J Amer med Ass* 191 (1965) 26
- 26 — ECONOMOU S G and SOUTHWICK W W Surgical management of Hodgkin's disease *Ann Surg* 148 (1958) 705
- 27 STRICKSTROCK K H MUSSHOFF K und BOUTIS L Methode zur zusätzlichen prophylaktischen Bestrahlung bei der Lymphogranulomatose *Strahlentherapie* 133 (1967) 337

REFERENCES

- 1 FRUDENBERG K. Grundriss der medizinischen Statistik. Schattauer Verlag Stuttgart 1962
- 2 HAMANN W., OEHLERT W., MUSSHOFF K. and NUSS A. Histologic classification of Hodgkin's disease and its prognostic validity
- 3 JACKSON JR H. and PARKER JR F. Hodgkin's disease and allied disorders. Oxford University Press New York 1947
- 4 KAPLAN H. S. Role of intensive radiotherapy in the management of Hodgkin's disease. Cancer 19 (1966) 356
- 5 — Radiothérapie de la maladie de Hodgkin à la Stanford University School of Medicine. Résultats joints. Nouv. Rev. franç. Hémat. 4 (1966) 73
- 6 — Technique statistique d'analyse des résultats de la radiothérapie de la maladie de Hodgkin. Nouv. Rev. franç. Hémat. 6 (1966) 115
- 7 — Evidence for a tumoricidal dose level in the radiotherapy of Hodgkin's disease. Cancer Res. 26 (1966) 1221
- 8 — Long term results of palliative and radical radiotherapy of Hodgkin's disease. Cancer Res. 26 (1966) 1250
- 9 LUKFS R. J. and BUTLER J. J. The pathology and nomenclature of Hodgkin's disease. Cancer Res. 26 (1966) 1063
- 10 — — and HICKS E. B. Natural history of Hodgkin's disease as related to its pathologic picture. Cancer 19 (1966) 317
- 11 MUSSHOFF K. und BOUTIS L. Die Frage der Heilbarkeit der Lymphogranulomatose beurteilt nach den Behandlungsergebnissen der Freiburger Medizinischen Klinik. Ver. dtsch. Ges. inn. Med. 73 (1967) 322
- 12 — — Therapy results of Hodgkin's disease Freiburg. Br. 1948—1966. Cancer 21 (1968) 110
- 13 — — Beurteilung Behandlungsgrundsätze und -ergebnisse bei der malignen Lymphogranulomatose. Strahlentherapie (1968) Sonderband 69 S. 59
- 14 — — Die Behandlungsergebnisse der malignen Lymphogranulomatose in Abhängigkeit von individuellen und krankheitsspezifischen Faktoren. Freiburger Ergebnisse 1948—1967. Klin. Wschr. 47 (1969) 93
- 15 — BUSCH K. M. und KAMINSKI H. Lymphogranulomatose (Morbus Hodgkin) mit Knochenbefall. Symptomatologie mit besonderer Berücksichtigung des Röntgenbildes. Therapie und Prognose. Ein Bericht über 66 Fälle (Freiburger Krankengut 1948—1961). Fortschr. Röntgenstr. 101 (1964) 117
- 16 — BOUTIS L., STRICKSTROCK K. H. und MERTEN D. Die Behandlung der Lymphogranulomatose. Dtsch. med. Wschr. 92 (1967) 1603
- 17 — REHNEMANN K. H., BOUTIS L. and AFRAN J. The differentiation of two different types of stage IV of Hodgkin's disease. Progress in lymphology II. Miami 1968
- 18 — — — Die extranoduläre Lymphogranulomatose (Hodgkin's disease). Diagnose, Therapie und Prognose bei zwei unterschiedlichen Formen des Organbells. Ein Beitrag zur Stadieneinteilung der Lymphogranulomatose. Fortschr. Röntgenstr. 109 (1968) 776
- 19 — STAMM H., LUMPEL G. und COSSEL K. Zur Prognose der Lymphogranulomatose. Klinisches Bild und Strahlentherapie. In: Beiträge der inneren Medizin S. 549. Herausgegeben von Keiderling. Schattauer Verlag Stuttgart 1964
- 20 OEHLERT W. und HAMANN W. Zur Histologie der potentiell geheilten Lymphogranulomatose Patienten im Freiburger Krankengut. Strahlentherapie (1968) Sonderband 69 S. 113



Age at the primary diagnosis in 69 patients and age on disappearance of the tumor in 63 patients with nasopharyngeal angiofibroma illustrated by cumulative distribution curves

varied from 7 to 32 years 80 % being between 10 and 21, with a mean age of 15 years (see accompanying diagram)

Symptoms Nasal obstruction was the most common symptom and occurred in sixty three patients (91 %), combined in forty one patients (59 %) with spontaneous bleeding, which was often profuse and necessitated medical attention. The patients seen before they were 14 years of age usually had a short history and in fourteen out of nineteen it did not exceed one year. Half the number of those examined after the age of 16 had a history of more than a year and the patient admitted for treatment at the age of 32 had his first symptoms 5 years previously.

Gradual expansion of the tumour was evident in fourteen patients from bulging of the cheek or exophthalmus. In one instance intracranial spread of the tumour was found post mortem. In fifty three patients the tumour was confined to the nasopharyngeal space sometimes with extension into the nasal cavities or the paranasal sinuses or both, as shown below in a total number of 68 patients (one case was unclassified) the tabulation giving the location and spread of the tumour

Nasopharyngeal and posterior region of the nasal cavities	29	(43 %)
Nasopharyngeal space nasal cavities and the paranasal sinuses or both	24	(35 %)
Nasopharyngeal space the orbital cavity the infratemporal fossa or both	14	(12 %)
Nasopharyngeal space and intracranial extension	1	(1 %)

JUVENILE NASOPHARYNGEAL ANGIOFIBROMA

A clinical study of 69 cases

by

BERTA JEREB, A ÅNGGÅRD and I BÄRID

Juvenile nasopharyngeal angiofibroma was defined by MARTIN *et coll* in 1948 as follows 'a specific highly vascular, non infiltrating essentially benign neoplasm, occurring in the nasopharynx or posterior nasal cavity of pubescent males. Symptomatically the tumour is characterized by nasal obstruction, repeated epistaxis and by progressive growth throughout the period of adolescence, with a tendency towards spontaneous regression at about the time of sexual maturity'. This description is still in line with current concepts of this uncommon growth but it is evident from the numerous articles relating to its etiology (14, 15), behaviour and treatment (1—10, 12—16) that some uncertainty still exists.

Material Our investigation was based on an analysis of 69 patients admitted to Radiumhemmet during the period 1919—1966 with a diagnosis of nasopharyngeal angiofibroma. In sixty one of these, the diagnosis was confirmed by histologic examination. All the patients were males, age at primary diagnosis

operation had previously been performed and all were referred for radiotherapy. Fifty patients had only radiotherapy (see Table 1)

Percutaneous roentgen treatment was, until about 1947, usually combined with the local application of a radium tube, or an interstitial needle implant in large bleeding tumours. A 5 g short distance radium unit was used in four patients, a ^{60}Co unit in three and the 16 MV roentgen beam from a betatron in one patient. Irradiation with the ^{60}Co unit and the betatron was administered through two opposite portals while up to five portals were used with conventional 170 to 200 kV radiotherapy. The eyes were always protected. The treatment was sometimes repeated up to five times over a 2 to 3 year period.

A retrospective calculation of the tumour dose (in rad) in the nasopharynx has been attempted. The figures are, of course only approximate especially where radium was applied locally. The relationship between the tumour dose and the type of therapy is presented in Table 1. In 74% of the patients receiving conventional roentgen irradiation the tumour dose in the nasopharynx was less than 3 000 rad. When roentgen irradiation was combined with local radium therapy a higher dose was usually obtained, and in 57% of the patients it exceeded 3 000 rad.

High irradiation doses were applied until 1957. Between 1919 and 1926 the dose to the nasopharynx exceeded 6 000 rad in six of nine patients and the tumour dose was more than 10 000 rad in three patients when radium was applied locally in combination with external irradiation. A tendency has arisen since 1957 to use lower doses (Table 2).

Results

Three of the sixty nine patients were lost to follow up since the last treatment they had been free from the condition. Two patients treated during the previous three years had small residual growths one and two years after admission respectively. One patient who was alive 16 years after the first treatment has not been seen since treatment.

Of the remaining sixty three patients, forty seven have been free from the disease for more than 10 years, ten for more than 5 years and six for more than one year as may be seen from the following tabulation.

Follow up period after recovery (years)	1	2	5	10	20	30	>40
Number of patients	2	4	10	18	13	14	2

Five patients died of intercurrent disease after 11 years with no signs of a growth. The mean time elapsing from the beginning of therapy to the disappearance of the tumour was 5 years, the average age being 20 years. As many as

Table 1

Methods of treatment in a total of 69 patients and tumour dose when radiotherapy was given

Tumour dose rad	Operation alone	Operation plus roent- gen therapy	Operation plus roent- gen therapy plus Ra*	Roentgen therapy alone	Roentgen therapy plus Ra	Roentgen therapy plus ope- ration	Total
No radiotherapy 3							3
<2 000	3		1	9		1	14
2 000—3 000	1		1	20	1	1	24
3 000—6 000	3		3	8	6		20
>6 000			2	1	5		8
Total	3	7	7	38	12	2	69

* Local application of radium

Table 2

Doses given at different periods in the treatment of a series of 66 patients

Treatment period in relation to present time	Number of patients receiving doses (in rad)				Total number of patients
	<2 000	2 000—3 000	3 000—6 000	>6 000	
More than 10 years ago	1	4	1	—	6
10—20 years ago	3	6	1	1	11
20—30	3	6	8	1	18
30—40	6	7	9	—	22
More than 40 years ago	1	1	1	6	9
Total	14	24	20	8	66

Methods of treatment Over the period 1919—1966 the attitude to juvenile angiofibroma has undergone a change. The clinical appearance of the tumour prompted attempts at therapy and all the patients admitted in the early decades received treatment. With the better understanding of the benign nature of the growth in recent years, however, a more cautious approach has been adopted, and this since 1957. Only five patients have been treated at Radiumhemmet in the interval, and after 1966 none at all.

Operation, as the sole form of treatment, was performed in three patients with small tumours, one of these required a further operation. Two patients were given radiotherapy prior to surgery. In fourteen patients, at least one

Table 3

Radiation damage occurring in relation to the treatment doses given

	<2 000 rad	2 000—3 000 rad	3 000—6 000 rad	>6 000 rad
Atrophic changes in skin or nasal mucosa	11	10	8	7
Cataract		1	1	
Osteonecrosis				1
Total	6/14 (42 %)	11/24 (45 %)	9/20 (45 %)	8/8 (100 %)

cavity and caused bulging of the right cheek and palate and slight exophthalmus. Biopsy confirmed the clinical diagnosis of nasopharyngeal angiofibroma.

Conventional roentgen treatment was instituted through four facial portals with an approximate dose of 1 000 rad to the nasopharynx for 2 weeks. Two months later enlargement of the tumour was noticed together with impairment of the vision of the right eye through atrophy of the optic nerve.

As treatment almost identical to the first one was without effect, operation through a right Denker approach was performed. The posterior and medial walls of the maxillary sinus were bulging forwards and laterally but there was no destruction of bone; there was expansive growth through the medial wall of the orbit into the ethmoid and sphenoid regions. The neoplasm was removed piecemeal with moderate bleeding but the patient died under the operation. Autopsy disclosed destruction in the ethmoid and sphenoid regions with extension of the tumour (1 cm × 1 cm) into the middle cranial fossa. There was no invasion of the dura. The diagnosis of nasopharyngeal angiofibroma was confirmed histologically.

Case 2 A 14-year-old boy was referred to Radiumhemmet in 1923 with nasal obstruction and epistaxis for 11 months. A tumour growing from the nasopharynx into the left nasal cavity and causing bulging of the cheek and exophthalmus was diagnosed as a juvenile nasopharyngeal angiofibroma.

Conventional roentgen treatment with a tumour dose of 1 300 rad to the pharynx was given for 6 weeks through four facial portals but with no noticeable improvement. Three months later a further 1 800 rad was administered by the same technique and in addition 900 rad was given to the nasopharynx with a 5 g radium unit through the same facial fields. Local radium was also applied on several occasions: about 2 000 rad intracavitarily to the nasopharynx and 1 700 rad as a needle implant. The last treatment was administered in 1926 when the tumour was large and there was bulging of the cheek and exophthalmus. The total dose to the nasopharyngeal region was calculated to be 7 900 rad.

The patient remained under observation and the tumour gradually diminished until 1933 when it had disappeared. Atrophic changes of the nasopharyngeal and nasal mucosa together with impairment of the vision of the left eye due to cataract of the lens had however developed. The patient's condition remained unchanged until 1958 when he developed left dacryocystitis. Conservative treatment and antibiotics were without effect. Sequestered bone fragments of the maxilla were removed at two operations but no improvement was achieved. The patient died in 1960 at the age of 51 from frontal osteitis and meningitis.

80 % of all the patients were free from the disease before they were 25 years old. The condition lasted longer in patients under 14 years of age than in those who were older, more treatments were then administered although the total tumour dose was usually lower. A higher tumour dose and a longer duration of the disease was the rule in patients with tumours spreading to the infratemporal fossa.

Of the fifty eight patients in whom the time between primary therapy and regression of the tumour could be established, forty nine had objective as well as subjective improvement within 10 months, when a tumour dose of about 2 000 rad had been given for 4 weeks. In the remaining patients, the period of regression was as long as one or two years. The tendency to bleeding usually diminished during radiotherapy.

Radiation damage in relation to the follow up time after the first treatment occurred as follows:

Follow up period (years)	10	11—20	21—30	31—40	>40
Patients with radiation damage	0/6 (0 %)	3/12 (25 %)	7/16 (43 %)	14/22 (63 %)	7/7 (100 %)

Radiation damage occurring in relation to the treatment doses given is recorded in Table 3. Atrophic changes of the nasal and nasopharyngeal mucosa, such as dryness, formation of crusts and chronic rhinitis, were present in thirty one patients, in two of whom the changes were quite severe, with a tendency towards recurrent spontaneous epistaxis. They were seen in sixteen of the patients (40 %) given less than 3 000 rad. The proportion of patients with such changes increased with the time after treatment and they occurred in all the seven patients followed for more than 40 years, though two of them had radiation doses to the nasopharynx of between 2 000 and 3 000 rad. All the patients in whom the tumour dose was at least 6 000 rad and those who received local radium treatment had signs of irradiation damage. Two patients undergoing radiotherapy developed cataract and one patient osteonecrosis. Malignant degeneration was not encountered.

Two patients died from complications in the treatment. It would seem to be of interest to report briefly on these:

Case reports

Case 1 This was a 7 year old boy admitted to Radiumhemmet in August 1946 who had had symptoms of progressive nasal obstruction for the two previous months. The nasopharyngeal space was occluded by a lobulated tumour which extended into the right nasal

disease and the low age of the patients radiotherapy is inadvisable. When treatment is necessary because of bleeding, nasal obstruction or excessive growth, a surgical procedure, though not necessarily a radical one, is to be preferred. Preoperative radiotherapy may be used in an attempt to reduce bleeding or the operative risk.

Cases of nasopharyngeal angiofibroma have been reported where no complications have followed the use of 6 000 rad to the nasopharynx (7). On the other hand, doses of 1 000 to 2 000 rad have been deemed sufficient (1, 12) and complications after 3 000 rad have been negligible (7). In our experience the tumour dose need not exceed 3 000 rad to be given over about 6 weeks.

SUMMARY

A series of 69 patients with juvenile nasopharyngeal angiofibroma admitted during 1919—1966 has been analysed. Surgical measures were taken in three of the patients and sixty six were given radiotherapy. The clinical features, treatment and results are discussed. The treatment of nasopharyngeal angiofibroma should be directed against manifestations such as bleeding, nasal obstruction and excessive growth. Surgery is the method of choice.

ZUSAMMENFASSUNG

Eine Serie von 69 Patienten mit nasopharyngealem Angiofibrom, die zwischen 1919 und 1966 behandelt wurde, wurde kritisch analysiert. Operation erfolgte in drei und Bestrahlung in sechsundsechzig Patienten. Das klinische Bild, die Behandlung und die Resultate werden diskutiert. Die Behandlung des nasopharyngealen Angiofibroms sollte nur symptomatisch sein, um die Blutung, die Nasenverstopfung und zu grosse Ausbreitung des Tumors zu verhindern. Die Operation ist die Methode der Wahl.

RÉSUMÉ

Les auteurs ont analysé une série de 69 malades atteints d'angio-fibrome naso-pharyngien du jeune, hospitalisés entre 1919 et 1963. Trois de ces malades ont été traités chirurgicalement et soixante-six par radiothérapie. Les auteurs étudient les caractères cliniques, le traitement et les résultats. Le traitement de l'angio-fibrome naso-pharyngien devrait être dirigé contre l'hémorragie, l'obstruction nasale et le volume tumoral excessif. La chirurgie est la méthode de choix.

REFERENCES

1. APOSTOL J. V. and FRAZELL E. L. Juvenile nasopharyngeal angiofibroma. A clinical study. *Cancer* 18 (1965) 869.
2. BHATTIA M. L. and MISHRA S. C. Intracranial extensions of juvenile angiofibroma of the nasopharynx. *J. Laryng.* 81 (1967) 1395.

Discussion

Isolated cases of nasopharyngeal angiofibroma have been reported in females (5, 7, 8, 13, 14, 17) and at advanced ages (5, 8, 17), but the correctness of the diagnosis in many of these patients has been challenged (1, 10, 12). As all our patients were males and were seen before the age of 30, and most of the patients reported in the literature have been young males, a diagnosis of this condition in a female, or at middle age or over, is open to question.

An inverse relationship between the growth of a nasopharyngeal angiofibroma and age has frequently been noticed (1, 5, 10, 12, 13, 14). In the present series the duration of the disease was longer where the tumour appeared before puberty. Furthermore, the tumour seemed to be more active with a short duration of symptoms. These findings suggest that the growth of the nasopharyngeal angiofibroma is stimulated by hormonal factors before and during puberty, and that once sexual maturity is reached this effect is abolished.

Indications, methods, hazards of radiation, surgical therapy and a combination treatment have been dealt with by various authors (5, 6, 8, 9, 10, 12, 19). All the methods used have proved successful and appear to have yielded practically equivalent results. Regression after radiation is slow to begin (7, 10) and the duration of the disease is usually longer (1, 10). In the series in which surgical treatment has been the chief approach (10) an 80% cure rate has been reported under the age of 20, in the present series this figure was not recorded in patients under the age of 25.

The frequency of irradiation complications increased with the time elapsing after treatment though the dose was higher for the patients treated before 1927 (see Table 3). Younger patients with a long life expectancy are more likely to contract irradiation damage. The risk of inducing a malignant tumour must also be taken into consideration.

Both radiotherapy and surgery have been causes of fatal outcome, usually through aggressive attempts at complete irradiation of the tumour (1, 5, 6, 8, 10, 12). Two of the patients in our series died from complications of the treatment, one after extensive surgery and the other after irradiation. In the former, the intracranial spread of the tumour led to primary optic atrophy, this has also been reported by other workers (2, 3, 13). As patients with intracranial spread are considered to be poor surgical risks (2, 5) polytomography and angiography should be performed preoperatively when optic atrophy is present.

The treatment of nasopharyngeal angiofibroma seems to be more hazardous than the condition itself and should be given only after careful deliberation, and then be focussed on the symptoms. In view of the benign nature of the

EVALUATION OF RADIATION TREATMENT OF PAINFUL CONDITIONS OF THE LOCOMOTOR SYSTEM

A double blind study

by

IAN GOLDIE BENGT ROSENGREN ERIK MOBERG and ERLAND HEDELIN

Inflammatory and degenerative changes in joints and adjacent structures often cause pain the exact mechanism by which this is elicited is not completely understood (BOVICA 1953) It has been suggested that oedema following the increased capillary permeability in inflammation may exert pressure on nerve endings and result in pain (LINDAHL & REXED 1950 OLSSON 1958 ASBOE HANSEN 1963 HEIMANN 1964 CABITZA 1965 SICUTERI 1965, BALLABIO 1965) Impairment of venous drainage from subchondral bone as part of the inflammatory reaction may also contribute to pain (HULTH 1969) The alleviation of pain that takes place after the administration of antiphlogistic drugs would to some extent support these opinions

A relationship may exist between increased hydrogen ion concentration and pain — as evident in inflammatory conditions (MENKIN 1937 ROPES et coll 1953 CUMMINGS et coll 1966 GOLDIE et coll 1969) REVICK et coll (1949) believed that local changes in damaged tissues may bring about a lowering of the nerve threshold for pain and that end organs ordinarily concerned with other

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- 3 — — and PRAKASH J Lateral extensions of nasopharyngeal angiofibroma J Laryng 81 (1967) 99
- 4 BUTLER M R, NAJIB A M and HANAFER W New surgical approach to nasopharyngeal angiofibromas Trans Amer Acad Ophthal Otolaryng 71 (1967) 92
- 5 CONLEY J HEALEY W V BLAUGRUND M and PERZIN K H Nasopharyngeal angiofibroma in the juvenile Surg Gynec Obstet 126 (1968) 825
- 6 FIGI F A and DAVIS R E The management of nasopharyngeal fibromas Laryngoscope 60 (1950) 794
- 7 FITZPATRICK P J The nasopharyngeal angiofibroma Clin Radiol 18 (1967) 62
- 8 HANDOLSA A FARID H and ELWI A M Nasopharyngeal fibroma clinicopathological study of 70 cases J Laryng 68 (1954), 647
- 9 HARKOVEN M and MALMIO K On the treatment of nasopharyngeal fibroma and its results Acta oto laryng (Stockh) (1948) Suppl No 67, p 33
- 10 HARMIA R Nasopharyngeal angiofibroma A clinical and histopathological study Acta oto laryng (Stockh) (1958) Suppl No 146
- 11 MANI R L and SMITH M F W Rhabdomyosarcoma simulating nasopharyngeal angiofibroma Oncology 22 (1968) 74
- 12 MARTIN H, EHRLICH H E and ABELS J C Juvenile nasopharyngeal angiofibroma Ann Surg 127 (1948) 513
- 13 MISRA R N and MISHRA S C Nasopharyngeal angiofibroma Proceedings of the 8th international congress of oto rhino laryngology Tokyo 1965 Excerpta med found Internat congr ser 113 Amsterdam 1965
- 14 NEW G B and FIGI F A Treatment of fibromas of the nasopharynx Report of thirty two cases Amer J Roentgenol 12 (1924) 340
- 15 OSBORN D A The so called juvenile angiofibroma of the nasopharynx J Laryng 73 (1959) 293
- 16 SCHIFF M Juvenile nasopharyngeal angiofibroma A theory of pathogenesis Laryngoscope 69 (1959) 981
- 17 SHAHEEN H B Nasopharyngeal fibroma J Laryng 45 (1930) 259
- 18 TAPIA ACUNA R Nasopharyngeal fibroma and its treatment Arch Otolaryng 64 (1956) 451

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- 4 BUTLER M R, NAHUM A M and HANAFER W New surgical approach to nasopharyngeal angiofibromas Trans Amer Acad Otolaryng 71 (1967) 97
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- 6 FIGI F A and DAVIS R E The management of nasopharyngeal fibromas Laryngoscope 60 (1950) 794
- 7 FITZPATRICK P J The nasopharyngeal angiofibroma Clin Radiol 18 (1967) 67
- 8 HANDOUSI A, FARID H and ELWI A M Nasopharyngeal fibroma clinicopathological study of 70 cases J Laryng 68 (1954) 647
- 9 HARKONEN M and MALMIO K On the treatment of nasopharyngeal fibroma and its results Acta oto laryng (Stockh) (1948) Suppl No 67 p 33
- 10 HARMA R Nasopharyngeal angiofibroma A clinical and histopathological study Acta oto laryng (Stockh) (1958) Suppl No 146
- 11 MANI R L and SMITH M F W Rhabdomyosarcoma simulating nasopharyngeal angiofibroma Oncology 22 (1968) 74
- 12 MARTIN H, EHRLICH H E and ABELS J C Juvenile nasopharyngeal angiofibroma Ann Surg 127 (1948) 513
- 13 MISRA R N and MISHRA S C Nasopharyngeal angiofibroma Proceedings of the 8th international congress of oto rhino laryngology Tokyo 1965 Excerpta med found Internat Congr ser 113 Amsterdam 1965
- 14 NEW G B and FIGI F A Treatment of fibromas of the nasopharynx Report of thirty two cases Amer J Roentgenol 12 (1924) 340
- 15 OSBORN D A The so called juvenile angiofibroma of the nasopharynx J Laryng 73 (1959) 293
- 16 SCHIFF M Juvenile nasopharyngeal angiofibroma A theory of pathogenesis Laryngoscope 69 (1959) 981
- 17 SHAHJHAN H II Nasopharyngeal fibroma J Laryng 45 (1930) 259
- 18 TAPIA ACUNA R Nasopharyngeal fibroma and its treatment Arch Otolaryng 64 (1956) 451

Table 1

Distribution according to age and sex in the two groups subjected and not subjected to radiation

	Group given radiation treatment			Group not receiving treatment		
	Men	Women	Total	Men	Women	Total
20—30	4	1	5	3	1	4
31—40	4	7	11	9	6	15
41—50	22	19	41	18	19	37
51—60	27	35	62	19	29	48
61—70	20	28	48	28	29	57
71—80	10	20	30	11	16	27
81—90	3	5	8	1	5	6
			205			194

having carried out no systematic investigation on the results of radiation treatment in joint disorders were nevertheless of the opinion that it may be of benefit LEQUESNE (1967) found that radiotherapy had no effect in osteo-arthritis of the hip joint except when there was an associated trochanteric bursitis

Evaluation of the results in a special treatment of inflammatory and degenerative joint disorders becomes difficult as the course is most variable. Moreover also the radiation treatment varies both with regard to fractionation and total dose. Some believe in smaller doses at repeated intervals (OGISNER *et coll* 1940 STOLL 1957 HULTBERG *et coll* 1963) e.g. 25 to 100 R a day for 3 to 5 days. Others find an initial treatment with up to 400 R daily with a total of 2 400 R in a week to be of greater benefit (DE LORIMIER 1937) and HORWITZ *et coll* (1944) suggested 250 R a week for 8 or 7 weeks.

Difficulties in the evaluation of results of a therapeutic agent may thus arise when the disease for which a patient is treated causes symptoms that at least for some time subside spontaneously and also when the mode by which the therapeutic agent is applied varies considerably. Controlled series have as yet not been encountered excepting one reported as a discussion by NEWELL in 1939 and one by PLECK in 1952. In the former series both in alternate cases were shielded from the radiation by a lead filter practically no difference in the clinical course of the whole series was apparent. PLECK reported no difference in the results of a treated and non treated controlled series of calcifying tendinitis of the shoulder. LINDAHL & BACKLUND (personal communication) in a double blind study recorded no difference in the results of treated and untreated groups with joint disorders.

The aim of the present study was to compare two groups of patients with

forms of sensations are altered in a way to cause the impulses originated to evoke the sensation of pain

LINDAHL (1961) has demonstrated the relationship between increased hydrogen ion concentration and severe pain in the skin, whereby the hydrogen ion may be the chemical mediator that triggers the pain stimulus in the nerve endings. Alkalinization alleviated pain.

Painful conditions of the locomotor system — possibly due to the above mechanism — have long been regarded suitable for radiation treatment. Proliferating tissue, such as endothelium in newly formed blood vessels in the subchondral region, and partly the region of the inflammatory reaction in the joint capsule at its junction with the bone cartilage, are sensitive to ionizing radiation. On the other hand, PFANDERGRASS *et coll* (1941) and ELLINGER (1957) stressed that irradiation transfers an area of passive hyperemia into active hyperemia which among other things decreases oedema and increases in lymphatic flow. White blood cells and particularly the lymphocytes are still more radiosensitive and react to low doses of one or a few hundred rad (TROWELL 1952).

Irradiation may cause slight initial acidosis which however is followed by a longstanding alkalosis (ELLINGER 1957). Another factor that is also influenced by irradiation is the calcium content of tissue. This is increased, which leads to a more marked anti-oedematous effect (ELLINGER 1957, HULTBERG *et coll* 1963).

Radiation treatment is supposed to counteract the establishment of certain strongholds for the maintenance of the inflammatory reaction that leads to pain in some common joint and juxta-articular disorders. Suitable doses for treatment of these diseases are considered to be in the region of a few hundred R and not over 1 000 R in one series. A total dose of up to about 2 000 R is considered to produce no disturbing late side effects, such as fibrosis and impairment of the mobility of the joint.

In the treatment of spondylosis deformans of the spine the doses have to be low due to the risk of neoplastic bone marrow disease (GRAHAM 1960).

The conception that pain relief can be attained has been widely accepted but little reported. SANDSTROM *et coll* (1937) gave details of successful treatment. OCHSNER *et coll* (1940) published the results in fifty-two patients with post-traumatic pain. There was relief of pain in thirteen out of twenty-seven post-fracture patients, in six out of fourteen posttraumatic patients without fracture, and in five out of eleven patients with joint pain following operation. Relief was therefore obtained in approximately 50%. HORWITZ *et coll* (1944) reported successful treatment in nine patients with hydroarthrosis of the knee joint. STOLL (1957) assessed the relief of pain in sixty-two patients receiving radiation therapy for posttraumatic arthrosis, para arthrosis and fasciitis. Alleviation was reached in 50% of patients within 1 to 4 weeks. HULTBERG *et coll* (1963), though

treated locomotor ailments amounting to 439 are given in Table 2. Roentgen examinations were performed in all the patients prior to the treatment in order to exclude malignant or other disease not suitable for this type of treatment.

All the patients were before the treatment individually interrogated and examined by an orthopaedic surgeon who did not know which patients were to receive treatment. The diagnosis was modified when it did not agree with the initial one. A radiologist selected the area to be treated. The patients in one of the groups were subjected on alternate days to a series of roentgen treatments according to a given procedure. Every second day this procedure was repeated for the other group of patients but then irradiation was prevented by a lead diaphragm. The procedure was repeated for both groups until conclusion of the treatment period. Neither the patient, nor the orthopaedic surgeon, the radiologist or the referring physician were to know who had received the true radiation treatment.

Six weeks following the conclusion of the treatment procedures, control of the patients was made by the orthopaedic surgeon who still did not know which patients had received irradiation. The interrogation was thus renewed and included post therapy results and an objective examination. The fee for treatment as decided by the National Health Insurance representative was paid by all patients irrespective of treatment or not. When the follow up study was terminated, the code for the treated and untreated patients was released and studied by the orthopaedic surgeon and the radiologist.

The following factors were applied in the radiation treatments: 170 kV, HVL 1 mm Cu, and usually 40 cm SSD.

The shoulders were irradiated with $10\text{ cm} \times 12\text{ cm}$ collimators. Two opposing beams were used with an exposure of $3 \times 150\text{ R}$. In acute cases the exposures were lower, i.e. 75 or 100 R. Knees were also irradiated with two opposing fields with the same collimators and $3 \times 200\text{ R}$. Hips were treated with three treatment fields: one ventral, one lateral and one dorsal, the field size being $9\text{ cm} \times 15\text{ cm}$. The SSD in these treatments was 50 cm and the exposure per field $3 \times 200\text{ R}$. Trochanteritis was treated with one ventral and one lateral field, applying the same field size and dose.

Patients with spondylosis deformans cervicalis, thoracalis et lumbalis were treated with two dorsal fields angled at 25 to 30° . In the cervical spine the collimators measured $4.5\text{ cm} \times 12\text{ cm}$ and in the thoracic and lumbar spine the fields were generally 11 cm wide and 10 to 15 cm long. The SSD for the cervical spine was 40 cm and for the thoracic and lumbar spine 50 cm. Every field was exposed to $3 \times 200\text{ R}$. Epicondylitis was treated with a field measuring $6\text{ cm} \times 8\text{ cm}$ and $4 \times 150\text{ R}$. Patients with calcaneodynia were treated with the same tubes and two opposing fields: one medial and one lateral, the exposure being $3 \times 150\text{ R}$.

Table 2

Diagnoses and number of patients in the two groups which received respectively true and 'false treatment for different disorders

	Radiation treatment	False radiation treatment	Total
<i>Spondylosis</i>			
Cervical	34	47	81
Thoracic	—	1	1
Lumbar	2	4	6
<i>Arthrosis (osteoarthritis)</i>			
Arthrosis def art hum scap (roentgen evidence of cartilage destruction of hum head)	3	7	10
Arthrosis def art acromioclav	3	6	9
Arthrosis def art cubiti	—	1	1
Arthrosis def art carpometacarp	5	1	6
Arthrosis def coxae	14	9	23
Arthrosis def genus	53	39	92
Arthrosis def art talocrur	2	2	4
Arthrosis def art metatarsophal	3	—	3
<i>Tendinitis</i>			
Peritendinitis hum scap	70	71	141 (5 acute cases)
Epichondylitis lat et med hum	11	14	25
Trochanteritis	4	2	6
Tendinitis lig coll regio genus	1	—	1
Calcaneodynia (spur and plantar bursitis)	5	4	9
<i>Synovitis</i>			
Genus	2	—	2
Metatarsophal	1	—	1
Other	10	8	18
			Total 439

ailments of the locomotor system, only one of which received radiation treatment using a double blind technique

Material and Methods

The series consists of 399 patients referred for disorders of the locomotor system. The age and sex distribution appear in Table 1 and the diagnoses of the

Table 4

Diagnoses and subjective and objective evaluations of the results in the group not exposed to radiation

	Total	Subjective evaluation		Objective evaluation		
		Improvement	Same or worse	Improvement	Same	Worse
Spondylosis cervicalis cervicorhizopathia	47	33	14	20	20	3
Spondylosis thoracalis						
Spondylosis lumbalis	5	1	4	1	4	—
Peritendinitis hum. scap.	71	47	24	38	19	7
Epicondylitis lateralis med. hum.	14	9	5	8	4	1
Arthrosis def. art. hum. scap.						
Arthrosis def. acromioclav.	13	10	3	9	2	—
Arthrosis def. art. cubitus	1	1	—	1	—	—
Arthrosis def. art. carpomet. carp.	1	1	—	1	—	—
Trochanteritis	2	1	1	—	1	—
Calcaneodynia	4	3	1	3	1	—
Tendinitis ischio-genus						
Synovitis genus	—	—	—	—	—	—
Synovitis metatarsophal.	—	—	—	—	—	—
Arthrosis def. coxae	9	5	4	1	6	—
Arthrosis def. genu	39	23	16	9	28	2
Arthrosis def. art. talocrur.						
Arthrosis def. art. metatarsophal.	2	1	1	1	1	—
Other	8	4	4	1	6	1
Total	216	139 (64%)	77 (36%)	93 (43%)	92 (43%)	14 (6%)

During all the treatments true or false, a dental roentgen film was placed in the beam as a check. It was of course blackened in all the true treatments and was unexposed in the false ones.

All the patients were directed not to take any antiphlogistic or other drugs nor physiotherapy for their ailments during the treatment period or during the subsequent follow up period.

Results and Discussion

A total of 393 out of the 399 patients were controlled. One patient had died and three could not be traced. The overall results with analyses of the diagnoses

Table 3

Diagnoses and subjective and objective evaluations of the results in the group treated with radiation

	Total	Subjective evaluation		Objective evaluation		
		Improvement	Same or worse	Improvement	Same	Worse
Spondylosis cervicalis						
cum rhizopathia	34	21	13	14	18	2
Spondylosis thoracalis						
Spondylosis lumbalis	2	1	1	1	1	—
Peritendinitis hum. scap.	70	52	18	31	39	4
Epicondylitis lat. et med. hum.	11	5	6	4	6	1
Arthrosis def. art. hum. scap.						
Arthrosis def. acromioclav.	6	5	1	3	3	—
Arthrosis def. art. cubiti	—	—	—	—	—	—
Arthrosis def. art. carpometacarp.	5	4	1	2	3	—
Trochanteritis	4	3	1	2	1	1
Calcaneodynia	5	4	1	3	2	—
Tendinitis regio. genis.						
Synovitis genis.	3	3	—	2	—	—
Synovitis metatarsophal.	1	—	1	—	1	—
Arthrosis def. coxae	14	6	8	4	7	2
Arthrosis def. genis.	53	37	16	22	30	1
Arthrosis def. art. talocrur.						
Arthrosis def. art. metatarsophal.	5	4	1	2	2	1
Other	10	7	3	5	1	—
Total	223	152 (68 %)	71 (32 %)	95 (43 %)	107 (48 %)	12 (5 %)

No collimators were used in other regions. The areas around the treatment fields were protected with lead rubber.

Osteoarthritis of the joints of the hand was irradiated with 3×150 R to one volar and one dorsal field. Osteoarthritis of the joints of the foot was treated in the same way with two opposing fields and 3×200 R. Osteoarthritis of the metatarsophalangeal joints was treated with one dorsal field and 4×150 R.

The depth doses calculated in the different treatment regions were approximately between 500 and 1 000 rad.

4) may be noted, however. In Table 3 the subjective improvement is 68 % and the objective improvement 43 %. In Table 4, the subjective improvement is 64 % and the objective improvement 43 %. The difference between the subjective evaluations in the two groups may to some extent depend on the failure of some patients to come to the interrogation and on the difficulty of obtaining a satisfactory anamnesis. However the recorded evaluations of subjective improvements are about the same for both groups of patients, exposed or not exposed to the radiation treatment and there is no difference between the two groups in the evaluations of objective improvements.

Our observations are further substantiated by the general individual opinions about the treatments. A strict correlation could be established between satisfied and subjective improvement as mentioned in Tables 3 and 4. In the group exposed to radiation 66 % satisfied correspond to 68 % subjective improvements in Table 3. In the group not exposed to radiation 63 % satisfied correspond to 64 % subjective improvements in Table 4. The results obtained correspond well with what is generally accepted by radiotherapists. HOWARD (1957) reported that 70 % of the patients with spondylarthrosis improved after radiation treatment.

A question commonly discussed is the time from final treatment to improvement. In this study improvement appeared to occur after 2 to 3 weeks both in the exposed and the unexposed groups (Table 5).

The tendency in the whole series as such was thus improvement after a few weeks whether radiation treatment was given or not. Further analysis of the three largest groups: humeroscapular peritendinitis, osteoarthritis of the knee and cervical spondylosis revealed that they together comprised 75 % of the total material or 157 patients in each of the exposed and unexposed groups. As for the subjective estimation of improvement in the exposed group it was observed in 74 % of humeroscapular peritendinitis, in 70 % of osteoarthritis of the knee and in 62 % of cervical spondylosis. With 68 % improvement in the total exposed group there is thus good correlation between the subgroups and the whole of the exposed group.

Corresponding observations were made as regards the objective evaluation: 44 % of humeroscapular peritendinitis, 42 % of osteoarthritis of the knee and 41 % of cervical spondylosis or 43 % of the total exposed group were improved.

As for the group not exposed to radiation treatment similar trends in the subjective estimation were registered. The improvement for humeroscapular peritendinitis was 66 %, for osteoarthritis of the knee 59 % and for cervical spondylosis 70 % or 64 % in the total series.

The objective evaluation revealed better results in humeroscapular peritendinitis where improvement occurred in 52 %, as compared to 43 % of the total un-

Table 5

Time for improvement following termination of treatment

	During treatment	Immediately after treatment	Weeks after treatment				
			1	2	3	4	5
Exposed to radiation	5	7	29	29	47	15	7
Not exposed to radiation	10	7	16	28	35	13	8

Table 6

Increased pain or other local reaction during treatment in relation to result of irradiation

	Pain during treatment	Subjective result after treatment	
		Improvement	Worse
Exposed to radiation	12	7	5
Not exposed to radiation	11	11	5

The discrepancy in the respective numbers of patients with subjective and objective evaluations is due to the failure of some patients to come to interrogation. The treatment, according to the patients' opinions, was satisfactory in 148 of those who had received radiation treatment and in 135 of those who had not been exposed to radiation, whereas dissatisfaction was recorded for 75 of the patients who had received radiation treatment and 81 of those who had not been exposed to radiation.

The time for improvement following the termination of the series of treatments is recorded in Table 5. A number of patients had some difficulty in giving accurate information and others stated that the pain had subsided gradually. Pain and local discomfort during the treatment period were also registered and related to the end results with a view to find out if these could have been influenced by the reactions (Table 6).

No true differences in the follow up results, with the doses used in this investigation, could be established between the group exposed and the group not exposed to radiation treatment.

Certain differences in the subjective and objective registrations (Tables 3 and

RÉSUMÉ

Les auteurs ont étudié une série de 399 malades correctement contrôlés et représentant 439 atteintes douloureuses de l'appareil locomoteur. Ils sont arrivés à la conclusion que le résultat final est le même chez les malades traités par radiothérapie pour ces affections et chez les malades qui n'ont pas subi ce traitement.

REFERENCES

- ASBOE HANSEN G. Connective tissue. *Ann Rev Physiol* 25 (1963) 41
- BALLABIO C. B. Clinical properties of Indomethacin. *Int Symp Non steroidal Antiinflammatory Drugs* p 342. Excerpta Med. Amsterdam 1965
- BONICA J. J. The management of pain. I ca & Febiger Philadelphia 1953
- BURITZ H. Beiträge zur pathologischen Physiologie der Entzündung 10 Über die Beeinflussung der traumatischen Entzündung durch Röntgenstrahlen. *Frankfurt Z. Path.* 44 (1932) 57
- CABITZA A. Benzindamine in orthopaedics. *Int Symp Non Steroidal Antiinflammatory Drugs* p 434. Excerpta Med. Amsterdam 1965
- CLUMINGS A. and NORDBY G. L. Measurements of synovial fluid pH in normal and arthritic knees. *Arthr. and Rheum.* 9 (1966) 47
- DE LORIMER A. A. Roentgen therapy in acute paronychia. *Amer J Roentg* vol 38 (1937) 178
- ELLINGER F. Medical radiation biology p 448. Charles C. Thomas Springfield Illinois 1957
- FRIGGNERO E., POPPE E. och ROMANUS R. Strålterapi pp 103-158 (In Swedish). Almquist & Wiksell Stockholm 1963
- FREUND F. Experimentelle Grundlagen der Röntgentherapie entzündlicher Prozesse. *Strahlentherapie* 40 (1931) 333
- GOLDIE I. and NACHEMSON A. Synovial pH in rheumatoid knee joints. I The effect of synovectomy. *Acta orthop scand.* 40 (1969) 634
- — Synovial pH in rheumatoid knee joints. II The effect of local steroid treatment. To be published in *Acta orthop scand.*
- GRAHAM D. C. Leukaemia following X-ray therapy for ankylosing spondylitis. *Arch intern Med* 105 (1960) 51
- HJERMAN P. Oxyfenbutazon i kirurgien. (In Swedish). *Nord Med* 71 (1964) 363
- HORWITZ I. and DILLMAN M. A. The effect of roentgen rays on the joint effusions in certain nonspecific articular lesions in humans and on the normal joints of dogs. A preliminary report. *Amer J Roentgenol* 51 (1944) 186
- HOWARD A. The value of irradiation in ankylosing spondylitis. *Brit J Radiol* 30 (1957) 371
- HULTBERG B., LANGREN O. and ROMANUS R. Inflammatoriska och degenerativa sjukdomar i mjukdelar och leder. (In Swedish). In *Strålterapi* p 158. Almquist & Wiksell Stockholm 1963
- HULTIN A. Osteoarthritis. The ageing of connective tissue. *Thule Int Symp* October 1968. Nordiska Bokhandels Förlag Stockholm 1969
- LEQUATRE M. Disease of the hip in adult life. *Folia Rheumatol Documenta Geigy* (1967)
- LUNDHIL O. Experimental skin pain. *Acta phys scand* 51 (1961) Suppl No 179
- Personal communication (1969)
- and REXED B. Histologic changes in spinal roots of operated cases of sciatica. *Acta orthop scand* 20 (1950) 215

exposed group Osteoarthritis of the knee was somewhat worse with only 23 % improvement, but cervical spondylosis with an improvement of 42 % corresponded well to the figure for the total group It has not been possible to find any obvious reason for this

There was no statistical difference with the χ^2 test in a comparison of the results in the exposed and the corresponding unexposed groups

The results of this investigation suggest that no difference exists in the end results of exposed or unexposed groups of patients to the generally adopted doses of radiation treatment in painful inflammatory and degenerative conditions of the locomotor system

Addendum in proofs

A questionnaire was sent out to 368 patients seven to eight months after the 6 week control Fifteen patients could not be traced owing to change of address or death The patients were asked if there had been any change in their condition since the last control and whether they had received any further treatment Of the 353 patients who received the questionnaire 325 replied

Further treatment had been given to seventeen patients in the treated improved group they had received physio and short wave therapy but no irradiation Fifteen of the patients in the treated not improved group had had further treatment excluding irradiation In the untreated improved group sixteen patients had received further treatment excluding irradiation and in the untreated not improved group eighteen patients had received further treatment but not irradiation

The results still indicated that no differences existed as regards the degree of improvement between the treated and untreated groups and that the results had not materially altered since the 6 week control In the treated group 104 patients of the 111 improved at the 6 week control were still improved at the 8 month control of the 47 not improved at the 6 week control 21 had improved at the 8 month control In the untreated group 95 of 108 remained improved and 19 of the 59 changed their statement from not improved to improved

SUMMARY

A series of 399 patients suitably controlled and representing 439 painful locomotor ailments, was studied The conclusion was reached that no difference existed in the end results of patients exposed and those not exposed to radiation treatment for the conditions

ZUSAMMENFASSUNG

Ein Material von 399 Patienten die an 439 schmerzhaften Erkrankungen des locomotorischen Systems litten wurde kritisch überprüft Es ergab sich dass die Strahlenbehandlung das Endresultat nicht beeinflusst

EFFECT OF SELECTIVE TUMOR HEATING ON THE LOCALIZATION OF ^{125}I FIBRINOGEN IN THE WALKER CARCINOMA 256

II Heating with microwaves

by

E. S. COPELAND and S. M. MICHAELSON

It is thought that the primary effect of microwave irradiation on mammalian tissues results from increased molecular vibration within these tissues i.e. microwave irradiation increases tissue temperature (MICHAELSON et coll 1961). CATER et coll (1964) have shown that microwave heating acts synergistically with radiation in destroying the rat hepatoma 223. CATER review the field of microwave radiation therapy of tumors revealing that few tumors can be destroyed by microwaves alone. Their heating effect may, however, like warm water, be able to increase ^{125}I fibrinogen localization so that ^{125}I β radiation can destroy tumor tissue. Microwaves should be nearly completely blocked by fine mesh copper wire screening (REYNOLDS 1961). Hence the body of a rat bearing the Walker tumor could presumably be satisfactorily shielded from the microwave beam and only the tumor with its overlying skin directly exposed to the heating effects of the

- MENKIN V and WARNER C R Studies on inflammation VIII Carbohydrate metabolism local acidosis and the cytological picture in inflammation *Amer J Pathol* 13 (1937) 25
- NEWELL R Discussion *Radiology* 32 (1939) 314
- OCHSNER H C and MUNFORD E B Roentgen therapy for painful conditions of the bones and joints *Radiology* 34 (1940), 444
- OLSSON S E The dynamic factor in spinal cord compression A study on dogs with special reference to cervical disc protrusions *J Neurosurg* 15 (1958) 308
- PENDERGRASS L P and HODES O J Roentgen irradiation in the treatment of inflammation *Amer J Roentgenol* 45 (1941) 74
- PLENK H P Calcifying tendinitis of the shoulder A critical study of the value of X ray therapy *Radiology* 59 (1952) 384
- REINHOLD H und SAUERBREY R Die Röntgentherapie des Schulter Arm Syndroms Epikondyliten an Schulter und Ellenbogen *Dtsch med Wschr* 86 (1961) 165
- REVICH E STOOPEN R FRENK E and REVICH R A The painful focus II *Bull Inst appl Biol* 1 (1949) 21
- ROPER M and BAUER W Synovial fluid changes in joint disease Harvard University Press Cambridge 1953
- RUBIN P and CASARETT G Clinical radiation pathology p 38 W B Saunders Co Philadelphia 1968
- SANDSTRÖM C und WAHLGREN T Beitrag zur Kenntnis der Peritendinitis calcarea (sogen Bursitis calcarea) speziell vom pathologisch histologischen Gesichtspunkt *Acta radiol* 18 (1937) 263
- SICUTERI F Indomethacin as a new vasoconstrictor and therapeutic agent in migraine *Int Symp Non Steroidal Antiinflammatory Drugs* p 335 Excerpta Med Amsterdam 1965
- STOLL H A X ray therapy in post traumatic arthritis pararthrits and fascitis *Med J Austr* 22 (1957) 868
- TROWFLI O A The sensitivity of lymphocytes to ionizing radiation *J Path Bact* 64 (1932) 687

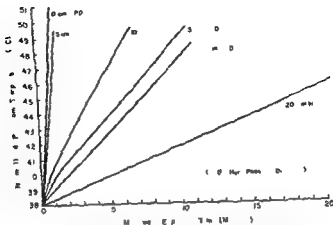


Fig. 2. Microwave dosimetry phantom study. The temperature of a 10 ml water phantom is plotted as a function of exposure time at various distances from the microwave transmitter waveguide horn. The microwave transmitter was operating at 110 kV and 180 mA. Approximate calculations suggest that at 15 cm HPD the effective microwave power concentration was 0.258 att/cm.

Materials and Methods

Experimental animals and materials were handled and prepared as previously described (COPELAND 1970).

Microwave irradiation technique. The microwave apparatus described by VERMAGEN (1961) was used for these irradiations (Fig. 1). Microwaves (continuous) of 10.7 cm wavelength and 2800 MHz frequency were produced by the transmitter operating at 1.19 kV and 180 mA. The irradiations were performed with the center of the tumor at a distance of 15 cm from the end of the waveguide horn i.e. 15 cm horn-tumor distance (HTD). Phantom studies were done to determine the effective power output at various distances from the horn. Ten grams of water were placed in a plastic vessel and the water temperature measured with a thermocouple as a function of irradiation time at various distances from the waveguide horn.

In order to shield the rat during irradiation a 2.5 centimeter hole was cut in a fine mesh wire screen which was wrapped around a lucite anesthesia chamber. The tumor was pulled outside the lucite cylinder and through the hole in the screen and thus selectively exposed to microwave irradiation (see Fig. 1). In order to determine the effectiveness of the wire screen as a shielding material against

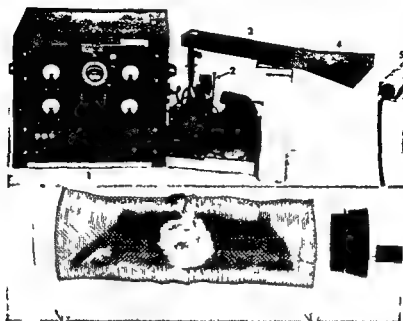


Fig 1 Microwave irradiation study apparatus. *Upper view*. Microwave irradiation unit (MPS 14 Radar set) (continuous wave). In the studies reported here the direct current power supply (1) was operated at 1.19 kV and 180 mA. Microwaves were generated by the magnetron tube (2) which was cooled by the centrifugal fan shown in right center. Microwaves left the magnetron tube entered a preliminary tunable waveguide, traveled along a coaxial cable to a stub antenna in the proximal end of the wave guide (3) and were then regenerated, they traveled down the wave guide to the horn (4) and thence to the tumor of the shielded rat (5). The microwaves had a frequency of 2,800 MHz and a wavelength of 10.7 cm (World War II S band). *Lower view*. Anesthesia chamber used for tumor microwave irradiation. This lucite cylinder was used as an anesthesia chamber for the microwave irradiation studies. During microwave irradiation the rat's body was shielded by the fine mesh wire screen shown here. As can be seen the tumor was exposed to microwaves by being pulled through a hole in the wire screen. Phantom studies showed that this wire screen effectively blocked microwaves.

microwave irradiation. This heating then should induce sufficient tumor damage to cause increased tumor ^{131}I fibrinogen localization.

BALE and co-workers have used radioiodinated human fibrinogen and anti fibrin antibody with limited success in the therapy of human tumors (DEWEY et coll 1963, McCARDLE et coll 1966). Some human tumors, like the Walker carcinoma 256 of the rat, do not show remarkable localizing ability for fibrinogen or antifibrin antibody. It is now obvious that in human cancer therapy, some method of getting greater and more rapid localization of radioactivity in tumor is needed if this technique is to be of widespread use.

The experimental objective of the present research is to determine whether sufficient injury can be imparted to tumor tissue by tumor directed microwave irradiation to enhance localization of iodinated fibrinogen in the Walker tumor.

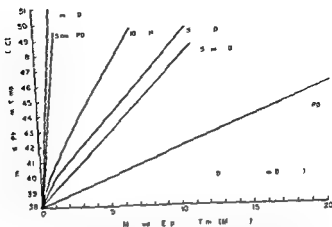


Fig. 2 Microwave dosimetry phantom study. The temperature of a 10 ml water phantom is plotted as a function of exposure time at various distances from the microwave transmitter wave guide horn. The microwave transmitter was operating at 110 kV and 180 mA. Approximate calculation suggests that at 15 cm HPD the effective microwave power concentration was 0.58 att/cm.

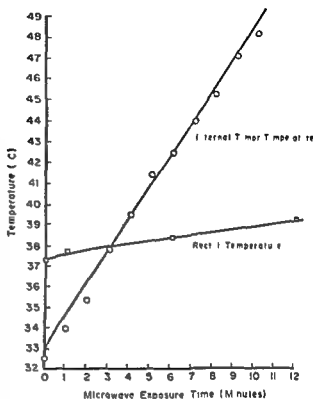
Materials and Methods

Experimental animals and materials were handled and prepared as previously described (COPELAND 1970).

Microwave irradiation technique. The microwave apparatus described by MERMAGEN (1961) was used for these irradiations (Fig. 1). Microwaves (continuous) of 10.7 cm wavelength and 2800 MHz frequency were produced by the transmitter operating at 119 kV, and 180 mA. The irradiations were performed with the center of the tumor at a distance of 15 cm from the end of the wave guide horn i.e. 15 cm horn tumor distance (HTD). Phantom studies were done to determine the effective power output at various distances from the horn. Ten grams of water were placed in a plastic vessel and the water temperature measured with a thermocouple as a function of irradiation time at various distances from the wave guide horn.

In order to shield the rat during irradiation a 2.5 centimeter hole was cut in a fine mesh wire screen which was wrapped around a lucite anesthesia chamber. The tumor was pulled outside the lucite cylinder and through the hole in the screen and thus selectively exposed to microwave irradiation (see Fig. 1). In order to determine the effectiveness of the wire screen as a shielding material against

Fig 3 Effect of tumor directed micro wave irradiation on tumor and rectal temperature This nearly linear temperature time curve was obtained when the tumor borne by a rat was irradiated with microwaves as described in the text and the internal temperature of the tumor was followed by a thermistor probe inserted into the center of the tumor The irradiation conditions (119 kV 180 mA 13 cm HTD) were the same used throughout the microwave studies The body of the rat was shielded with fine mesh copper wire screen as previously described The rectal temperature plotted here was measured with a rectal thermistor probe during the course of a typical irradiation treatment



microwaves, the water phantom was exposed to the microwave beam and the temperature was recorded as described above. Then the wire screen was inserted between the microwave source and the phantom, 4 cm from the phantom.

Results

The parameters measured, percentage of injected dose per gram tumor normalized ($\% \text{ID/gTN}$), therapeutic ratio, and percentage of whole body radio activity retained, were described in the preceding paper (COPELAND).

Microwave dosimetry. In order to determine the microwave power density and heating characteristics in the various regions around the wave guide horn dosimetry studies were conducted. The object of this study was to find a suitable irradiation distance, i.e. a horn-tumor distance at which the microwave power output was great enough that the tumor could be heated to about 45°C in a period of time short enough to be practical, but long enough to be easily controlled and reproduced. It also had to be demonstrated that fine mesh copper screen could effectively block microwave irradiation.

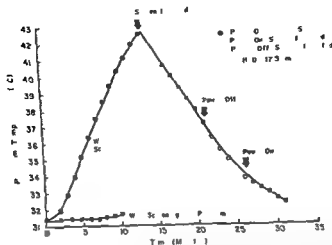


Fig 4 Effectiveness of fine mesh copper screen as a microwave shield. In order to determine the effectiveness of the wire screen as a shielding material against microwaves the water phantom was exposed to a microwave beam and the temperature was recorded as previously described. Then the wire screen was inserted between the microwave source and the phantom 4 cm from the phantom. As indicated in the figure no matter whether the microwave source was turned off or on the phantom temperature continued to drop at a nearly constant rate about $0.7^{\circ}\text{C}/\text{min}$. This study suggests that there is no heating effect of microwaves behind the wire screen. If the power was turned on after the wire screen was inserted there was little rise in phantom temperature during ten minutes of irradiation.

The rate of temperature increase of the 10 ml water phantom when placed at various distances from the wave guide horn is shown in Fig 2. At 15 cm HPD the temperature rose 5.75°C in 5 minutes. It is indicated in Fig 4 that the rate at which the phantom loses heat in this temperature range is about $0.7^{\circ}\text{C}/\text{min}$. Hence the phantom absorbed $18.5\text{ g cal}/\text{min}$. The phantom cross sectional area was 5.0 cm^2 thus the effective microwave power concentration at 15 cm HPD was $0.258\text{ watt}/\text{cm}^2$.

The results of a study in which a thermistor probe was inserted into the center of a 10 day Walker tumor borne by an anesthetized and shielded rat and the internal tumor temperature was recorded as a function of the time exposed to microwaves at 15 cm HTD are presented in Fig 3. This temperature rose 15°C in 10 minutes. Rat core temperature rose less than 2°C . The tumor phantom temperature rose 11°C when irradiated under the same conditions (see Fig 2). Such agreement between tumor and phantom temperature response is good in view of the highly greater cross-sectional area and lower heat loss rate of the tumor.

Table 1

Effect of tumor directed microwave irradiation on the localization of ^{131}I fibrinogen in the Walker carcinoma 256

Treatment	Number of rats	%ID/gTN	Therapeutic ratio	3 day whole body radioactivity retained (%)	Average rat weight (gram)	Average tumor weight (gram)
Control						
RT 10	14	0.81 ± 0.07	8.0 ± 0.9	19.0 ± 1.6	138 ± 5	15.04 ± 1.4
MW 5	10	$2.19 \pm 0.37^*$	$27.2 \pm 5.6^*$	23.8 ± 2.7	141 ± 3	12.08 ± 1.90
MW 10	10	$3.26 \pm 0.43^*$	$44.8 \pm 7.7^*$	24.9 ± 4.1	141 ± 3	11.97 ± 0.93
MW 12	5	$2.58 \pm 0.28^*$	$32.6 \pm 5.0^*$	16.6 ± 1.4	129 ± 3	5.39 ± 1.14

* Indicates a statistically significant difference from the control level at the 0.05 significance level

RT — indicates room temperature about 25 °C

MW is used here and elsewhere in this study as an abbreviation for microwave irradiation

On this and each of the following tables each parameter is followed by \pm standard error of the mean. The text should be consulted for a description of the experimental protocol used to obtain these data.

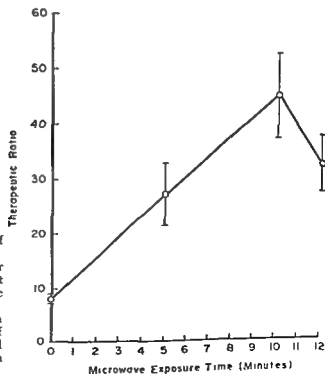


Fig 5 Effect of tumor directed microwave irradiation on the localization of ^{131}I fibrinogen in the Walker carcinoma 256. The therapeutic ratio for each of the three microwave treatment groups and the control is plotted here as a function of the microwave exposure time. The vertical bar at each point represents the standard error of the mean. All treatment groups showed a statistically significant difference from the control therapeutic ratio.

Table 2

Kinetics of tumor fibrinogen localization after selective tumor microwave irradiation

Treatment	Number of rats	ID/gTN	Therapeutic ratio	Whole body radioactivity retained (%)	Average rat weight (gram)	Average tumor weight (gram)
Control (R T 10) 24 hours	4	137±0.08	33±0.3	46.4±3.4	141±6	8.83±1.24
Control 47 hours	3	100±0.04	53±0.5	24.4±0.6	141±8	11.98±0.66
Control 67 hours	14	0.81±0.07	80±0.9	19.0±1.6	138±5	15.04±1.45
5 MW 21 hours	4	2.90±0.41	9.0±1.8	47.7±3.8	145±3	8.52±1.38
5 MW 47 hours	4	2.50±0.50	21.0±2.0	23.1±3.1	139±8	7.46±1.72
5 MW 67 hours	10	2.19±0.37	27.2±5.6	23.8±2.7	141±3	12.08±1.90
10 MW 21 hours	4	9.36±0.08	4.6±0.7	69.5±7.2	136±3	8.54±2.26
10 MW 47 hours	3	2.13±0.13	10.4±2.9	31.4±7.4	136±9	6.39±1.61
10 MW 67 hours	10	3.26±0.43	44.8±7.7	24.9±4.4	144±3	6.97±0.93

The text should be consulted for an explanation of the experimental protocol from which these data were obtained. All the 3-day (67 hrs) parameters reported here represent a compilation from several experiments and were reported in Table 1.

The results of a study demonstrating the effectiveness of copper wire screen as a microwave shield are presented in Fig. 4. The temperature record shows that there is no microwave heating effect behind the wire screen.

Effect of microwave irradiation on tumor fibrinogen localization. Experimental animals were treated in the manner previously described (COPELAND); their tumors being exposed to microwaves at 15 cm HTD for 5, 10 or 12 minutes. The tumors of control animals were mock irradiated i.e. positioned for irradiation but given none. The results of several microwave studies are compiled in Table 1. Both the parameters which measure fibrinogen localization indicated a statistically significant increase in localization above the control level. The ratios of experimental therapeutic ratio to control therapeutic ratio are 3.4, 5.6 and 5.1 for the 5, 10 and 12 minutes microwave exposure times respectively. The therapeutic

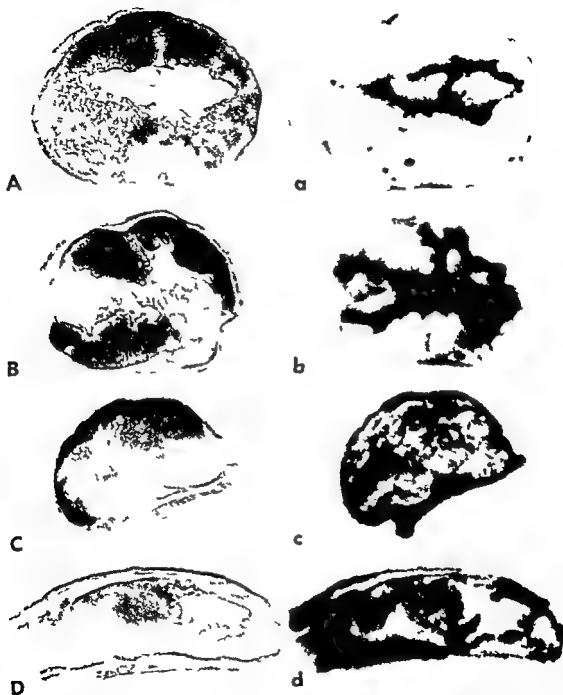


Fig. 6 Photomicrographs and autoradiographs of tumors electively irradiated with microwaves 3 day uptake magnification $\times 16$

A—*a*) Control tumor (RT 10). This tumor has a moderately sized pink stained medullary region and a large dark blue stained cortex. It is similar in all respects to previously described control tumors. Radioactivity is located primarily in the pink medulla.

B—*b*) Treated tumor (Microwaves). An enlarged pink stained medullary region is present in this tumor. Radioactivity is found in this region. The kinetic study reveals a more rapid third day localization pattern.

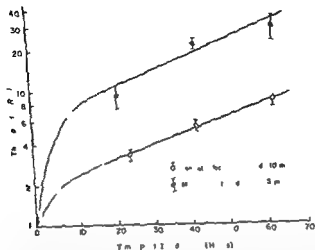


Fig. 7 Kinetics of ^{125}I -fibrinogen localization in the Walker carcinoma 256 after selective microwave irradiation of the tumor. Therapeutic ratio is plotted as a function of time between initiation of treatment and sacrifice for control and five minute microwave irradiation. The vertical bars at each point indicate the variation allowed by the standard error of the mean. In each group the therapeutic ratio seems to increase exponentially with time after about 20 hours post irradiation. All data plotted here were taken from Table 2. Curves to the left of the one day values are dotted to indicate that they are hypothetical.

ratio is plotted against microwave exposure time in Fig. 5. Representative stained sections and the corresponding radioautographs are presented in Fig. 6. In general, as the microwave exposure time is increased, the fraction of the tumor which is necrotic increases and the radioautograph density increases correspondingly. However, at the 5 minutes exposure level, radioautograph density is not limited to necrotic tumor tissue but encroaches into viable tissue zones. Although the 10 and 12 minutes exposed tumors do not appear to be completely necrotic, ^{125}I activity is distributed more or less evenly throughout the tumor. Other tumor sections not presented here reveal considerable tumor erosion at the 10 and 12 minutes exposure levels.

C—c) Treated tumor (Microwaves 10). This tumor does not show a characteristic medullary region. It seems to contain more vascular components than A—a or B—b. The whole section is stained pinkish blue. Radioactivity is deposited throughout the tumor.

D—d) Treated tumor (Microwaves 12). Like the preceding tumor section, no medullary region as such is present here. The section is pink in gross appearance but contains large regions of both wise normal tumor cells. The one region not containing radioactivity has a lard-like composition of cells with fragmented nuclei. Considerable tumor erosion was evidenced in other members of this group.

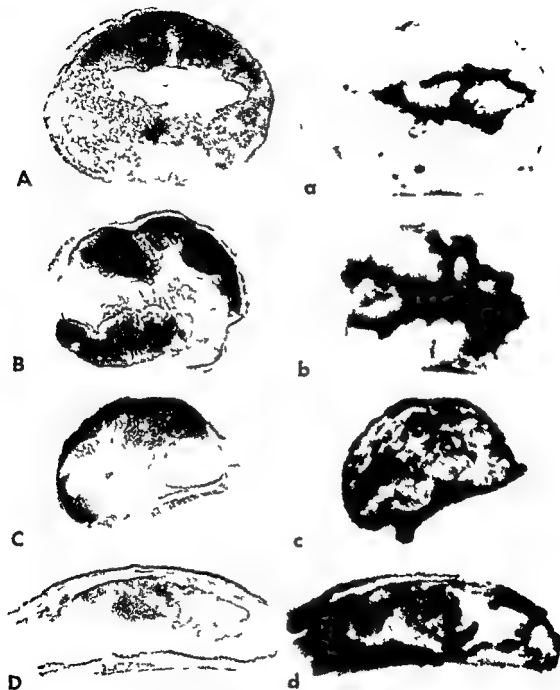


Fig. 6. Photomicrographs and autoradiographs of tumors selectively irradiated with microwaves 3 day uptake magnification $\times 16$.

A—a) Control tumor (R 110). This tumor has a moderately sized pink stained medullary region and a large dark blue stained cortex. It is similar in all respects to previously described 3 day control tumors. Radioactivity is located primarily in the pink medulla.

B—b) Treated tumor (Microwaves). An enlarged pink stained medullary region is present in this tumor. Radioactivity is found in this region. The kinetic study reveals events which precede this third day localization pattern.

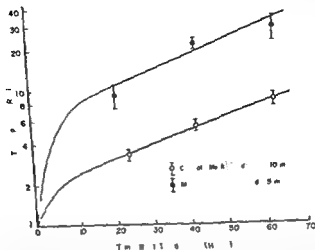


Fig 7 Kinetics of ^{125}I fibrinogen localization in the Walker carcinoma 256 after selective microwave irradiation of the tumor. Therapeutic ratio is plotted as a function of time between initiation of treatment and sacrifice for control and five minute microwave irradiation. The vertical bars at each point indicate the variation allowed by the standard error of the mean. In each group the therapeutic ratio seems to increase exponentially with time after about 20 hours post irradiation. All data plotted here were taken from Table 2. Curves to the left of the one day values are dotted to indicate that they are hypothetical.

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C—c) Treated tumor (Microwaves 10). This tumor does not show a characteristic medullary region. It seems to contain more vascular components than A—a or B—b. The whole section is stained pinkish blue. Radioactivity is deposited throughout the tumor.

D—d) Treated tumor (Microwaves 12). Like the preceding tumor section, no medullary region as such is present here. The section is pink in gross appearance but contains large regions of otherwise normal tumor cells. The one region not containing radioactivity has a border composed of cells with fragmented nuclei. Considerable tumor erosion was evidenced in other members of this group.

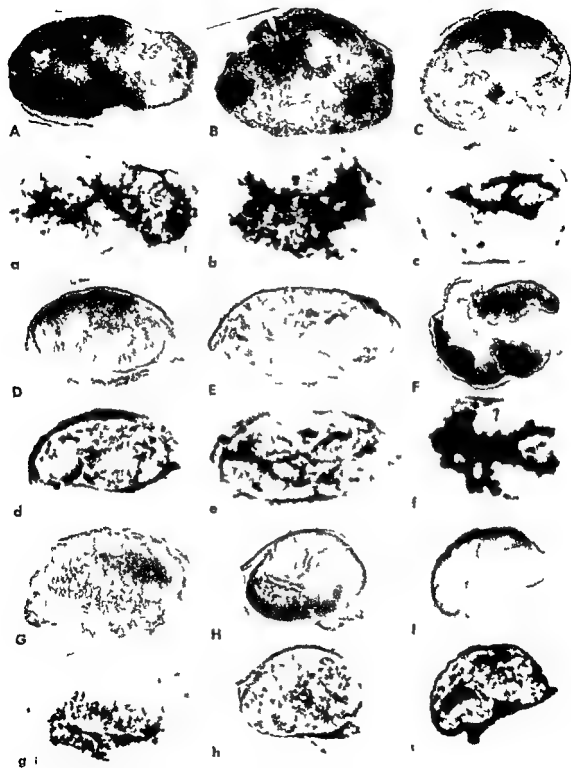


FIG. 11 (For legend see opposite page 1)

Kinetics of tumor fibrinogen localization after microwave irradiation The following experiment was performed to determine the time sequence of fibrinogen localization in microwave irradiated tumors. Rats were divided into three treatment groups and their tumors were either mock irradiated (controls) or exposed to microwave for 5 minutes or for 10 minutes. The irradiation conditions were identical to those used in the 3 day uptake study. Animals from each of the three groups were sacrificed on each of the three days following treatment. The data obtained from this study are presented in Table 2. The variation of the experimental and control therapeutic ratios as functions of time are plotted in Fig. 7. Representative stained sections and their corresponding radioautographs are presented in Fig. 8. In the control tumors ^{125}I activity is found in necrotic tumor tissue. At the 10 minutes microwave exposure level ^{125}I activity is found in the necrotic tumor medulla on the first day but is located throughout the tumor by the third day post treatment.

Discussion

It has been found that when the tumor is exposed to 2 800 MHz (CW) 0.258 watt/cm microwaves for five minutes localization of ^{125}I fibrinogen within the tumor is more than doubled for the next 3 days when compared to that in control tumors which were poisoned for irradiation but given none.

Kinetic studies carried out with the five minute microwave treatment group showed that tumor radioactivity reached its maximum within 24 hours after treatment and remained high for at least 3 days. These studies also suggest that the therapeutic ratio increased exponentially with time after about 20 hours following treatment initiation. A theoretical analysis of tumor fibrinogen local-

Fig. 8. Photomicrographs and radioautographs from kinetics of uptake study after microwave irradiation of tumor. Magnification $\times 1$.

A-a), B-b) and C-c) Control tumors (RT 10) 1st and 3 days. Radioactivity remains localized in the variously shaped tumor medulla. These control tumors have all the characteristics previously described for untreated tumors. There is no meaningful change in the pattern of radioactivity localization with time.

D-d), E-e) and F-f) Treated tumors (Microwaves 5) 1st and 3 days. On the first and second days after treatment (D-d) and E-e) the tumor sections have a generalized pinkish bluish tinge and do not have a characteristic medullary region. The third day section (F-f) has a pinkish bluish medulla. Radioactivity seems widespread but highest in the cortical regions on day 1. It is still widespread but more concentrated in the central regions on day 2 and definitely localized to the tumor medulla on day 3.

G-g), H-h) and I-i) Treated tumors (Microwaves 10) 1st, 2 and 3 days. A definite medullary region is evident on the first day after treatment but is not evident thereafter. On the first day radioactivity is limited to the pink medullary portion. On the second day radioactivity has become widespread throughout the pinkish blue tumor section. By the third day radioactivity has become quite concentrated throughout the tumor.

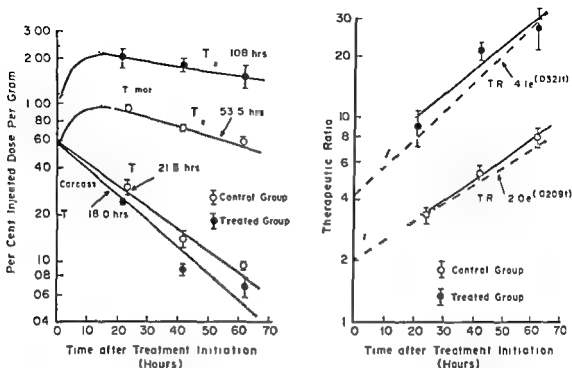


Fig. 9 Theoretical analysis of change in therapeutic ratio with time. *Left*: Specific activity of tumor and carcass is plotted versus time after treatment and the biological half lives of ^{131}I fibrinogen (T_{I}) are determined. *Right*: Experimental (solid lines) and calculated (dashed lines) therapeutic ratios are plotted versus time.

zation after five minutes of microwave irradiation is presented in Fig. 9. On the left of the figure, the logarithms of percentage of injected dose per gram (% ID/g) of irradiated and control tumor tissue and for the carcasses of treated and control rats are plotted against time. These curves are consistent with the hypothesis that ^{131}I fibrinogen is deposited in the tumor primarily during the first 20 hours after injection whether the tumor is irradiated or not, and that thereafter tumor radioactivity concentration decreases exponentially with time.

The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor. The initial carcass radioactivity concentration is approximately the same in the treated and control groups and the carcass biological half life of ^{131}I fibrinogen is almost the same in the two groups.

The straight line portions of the log % ID/g versus time plots can be assigned mathematical descriptions based on the exponential relation, $A_t = A_0 e^{-\lambda t}$, as in the previous study (COPELAND).

When the suitable constants determined in Fig. 9 are substituted into this rela-

tion the therapeutic ratio as a function of time for control and treated groups can be calculated

$$I R_{\text{control}} = 2.1 e^{0.193t} \quad I R_{\text{treated}} = 4.1 e^{0.099t}$$

These calculated expressions for the therapeutic ratios are plotted as functions of time on the right side of Fig. 9. The variation of the experimental therapeutic ratios with time are also plotted here as they were in Fig. 7. The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference in biological half life of ^{125}I fibrinogen in the tumor and carcass.

The effect of microwave treatment on total ^{125}I radiation doses to tumor and carcass can be calculated as in the previous paper

Thus

$$\frac{\text{average beta dose to tumor}}{\text{average beta dose to carcass}} = \frac{4.1 \times 4.19}{2.0 \times 2.17} = 17.2 \text{ for treated group}$$

$$= 4.3 \text{ for control group}$$

This calculation indicates that five minute microwave irradiation improved the ^{125}I fibrinogen radiation therapy of the Walker tumor nearly 400 per cent. In tumors whose positions can be reasonably well determined as by ^{125}I antifibrin antibody (McGARDLE et coll.) or classical diagnostic techniques tumor directed microwave irradiation seems an effective means to increase ^{125}I fibrinogen deposition to levels high enough for therapy. As shown previously (COPLAND) normal tissues in the tumor environment should not be damaged by heating.

Acknowledgement

This work is part of a Ph.D. thesis in Radiation Biology at the University of Rochester and was supported in part by a National Institutes of Health training grant in biophysics by RADC Contract AF30 (603) 224 U.S. Air Force dated 1 March 1958 and by Contract W 7401 Eng 49 U.S. Atomic Energy Commission. The publication has been assigned Report No. LR 149 126f.

SUMMARY

Heating of Walker carcinoma 256 by selective irradiation with 2800 MHz, 0.25 watt/cm microwaves for five minutes induced a substantial increase in the amount of ultravenously injected ^{125}I fibrinogen which will localize in the tumor. In irradiated tumors radioactivity was widely distributed and seemed to coincide with histologically demonstrable areas of tumor damage. This tumor heating technique could potentially increase tumor radiation therapy dose from 125 I fibrinogen 400 per cent.

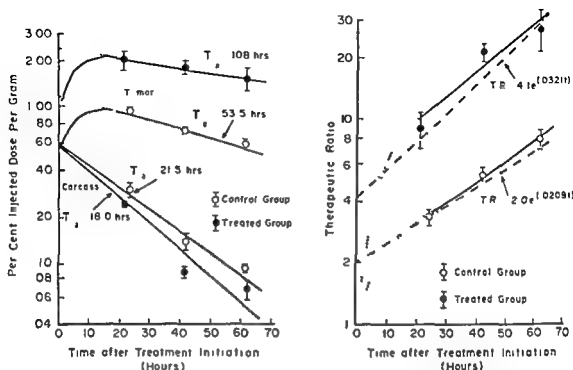


Fig. 9 Theoretical analysis of change in therapeutic ratio with time. Left: Specific activity of tumor and carcass is plotted versus time after treatment and the biological half lives of ^{131}I fibrinogen ($T_{1/2}$) are determined. Right: Experimental (solid lines) and calculated (dashed lines) therapeutic ratios are plotted versus time.

zation after five minutes of microwave irradiation is presented in Fig. 9. On the left of the figure, the logarithms of percentage of injected dose per gram (% ID/g) of irradiated and control tumor tissue and for the carcasses of treated and control rats are plotted against time. These curves are consistent with the hypothesis that ^{131}I fibrinogen is deposited in the tumor primarily during the first 20 hours after injection whether the tumor is irradiated or not, and that thereafter tumor radioactivity concentration decreases exponentially with time.

The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor. The initial carcass radioactivity concentration is approximately the same in the treated and control groups and the carcass biological half life of ^{131}I fibrinogen is almost the same in the two groups.

The straight line portions of the log % ID/g versus time plots can be assigned mathematical descriptions based on the exponential relation, $A_t = A_0 e^{-\lambda t}$, as in the previous study (COPELAND).

When the suitable constants determined in Fig. 9 are substituted into this rela-

tion the therapeutic ratio as a function of time for control and treated groups can be calculated

$$T R_{\text{control}} = 2.1 e^{0.193t}, T R_{\text{treated}} = 4.1 e^{0.09t}$$

These calculated expressions for the therapeutic ratios are plotted as functions of time on the right side of Fig. 9. The variation of the experimental therapeutic ratios with time are also plotted here as they were in Fig. 7. The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference in biological half life of ^{131}I fibrinogen in the tumor and carcass.

The effect of microwave treatment on total ^{131}I radiation doses to tumor and carcass can be calculated as in the previous paper

$$\begin{aligned} \frac{\text{average beta dose to tumor}}{\text{average beta dose to carcass}} &= \frac{4.1 \times 4.19}{2.0 \times 2.17} = 17.2 \text{ for treated group} \\ &= 4.3 \text{ for control group} \end{aligned}$$

This calculation indicates that five minute microwave irradiation improved the ^{131}I fibrinogen radiation therapy of the Walker tumor nearly 400 per cent. In tumors whose positions can be reasonably well determined as by ^{131}I antifibrin antibody (McGARDLE et coll.) or classical diagnostic techniques, tumor directed microwave irradiation seems an effective means to increase ^{131}I fibrinogen deposition to levels high enough for therapy. As shown previously (COPELAND) normal tissues in the tumor environment should not be damaged by heating.

Acknowledgement

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SUMMARY

Heating of Walker carcinoma 256 by selective irradiation with 2800 MHz 0.258 watt/cm microwaves for five minutes induced a substantial increase in the amount of intravenously injected ^{131}I fibrinogen which will localize in the tumor. In irradiated tumors radioactivity was widely distributed and seemed to coincide with histologically demonstrable areas of tumor damage. This tumor heating technique could potentially increase tumor radiation therapy dose from ^{131}I fibrinogen 400 per cent.

ZUSAMMENFASSUNG

Eine Erwärmung des Walker Carcinoms 256 durch selektive Bestrahlung mit 2 800 MHz, 0 258 Watt/cm² Mikrowellen 5 Minuten lang führt zu einem kräftigen Anstieg der Menge intravenös injizierten ¹³¹I Fibrinogens die sich im Tumor ansammelt In bestrahlten Tumoren ist die Radioaktivität weit verteilt und scheint mit der histologisch nachweisbaren Ausbreitung der Tumorschädigung übereinzustimmen Diese Wärmebehandlung des Tumors konnte möglicherweise die therapeutische Strahlendosis des Tumors durch ¹³¹I Fibrinogen um 400 % erhöhen

RÉSUMÉ

L'élévation de température dans un carcinome 256 de Walker par irradiation sélective de micro ondes de 2 800 MHz avec une puissance de 0 258 watt/cm² pendant cinq minutes augmente de façon importante la fixation dans la tumeur du fibrinogène marqué par ¹³¹I injecté par voie veineuse Dans les tumeurs irradiées la radio activité était largement dispersée et paraissait coïncider avec des zones où l'histologie montrait les lésions causées à la tumeur Cette technique de chauffage des tumeurs pourrait être quadrupler la dose de radiothérapie fournie par le fibrinogène marqué par ¹³¹I

REFERENCES

- CATER D B, SILVER I A and WATKINSON D A: Combined therapy with 220 kV roentgen and 10 cm microwave heating in rat hepatoma *Acta radiol Ther Phys Biol* 2 (1964) 321
- COPELAND E S: Effect of selective tumor heating on the localization of ¹³¹I fibrinogen in the Walker carcinoma 256 I Heating by immersion in warm water *Acta radiol Ther Phys Biol* 9 (1970) 205
- DEWEY W C, BAIR W F, ROSE R G and MARRACK D: Localization of antifibrin antibodies in human tumors *Acta Un int Cancr* 19 (1963) 185
- MCCARDLE R J, HARPER P V, SPAR I L et coll: Studies with Iodine 131 labeled antibody to human fibrinogen for diagnosis and therapy of tumors *J Nucl Med* 7 (1966) 837
- MERMAGEN H: Phantom experiments with microwaves at the University of Rochester *In Biological effects of microwave irradiation* p 143 Edited by M Peyton Plenum Press New York 1961
- MICHAELSON S M, THOMSON R A L and HOWLAND J W: Physiologic aspects of microwave irradiation of mammals *Amer J Physiol* 201 (1961) 351
- REYNOLDS M R: Development of a garment for protection of personnel working in high power R F environments *In Biological effects of microwave irradiation* p 71 Edited by M Peyton Plenum Press New York 1961

MULTIPLICATION OF TUMOR CELLS IN VITRO AFTER OXYIC OR ANOXYIC EXPOSURE TO ROENTGEN RADIATION

by

BO LITTEBRAND

The oxygen gradients in tumours vary. The radiation response of more or less hypoxic cells has consequently often been investigated with a view to improving the control of these cells by therapeutic irradiation. The study of radiation damage to mammalian cells grown in vitro represents a frequently practised experimental approach. The damage has been measured either by the inhibition of single cells to form colonies or by the inhibition of the growth of the cell populations. In the former instance data have accumulated on the reactions to single or multiple radiation exposures under oxyc, hypoxic and anoxic conditions (ELKIND & WHITMORE 1967). However, while many articles have dealt with the effect of single oxyc radiation exposures with growth inhibition as a criterion for the damage, few and incomplete data are available on the effect of multiple oxyc exposures or exposures in anoxia.

The purpose of the present experiments was to obtain information on the multiplication of cells irradiated with a single dose or the same dose split into two fractions in the presence or absence of oxygen. The growth curves will be analysed with regard to the clonogenic survival of the cells. Cultures of the ELD

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ascites tumour and several of its substrains with different radiosensitivity were therefore chosen as the biologic material for which data on the oxic and anoxic survival are available from previous studies (REVESZ & LITTBAND 1964 and 1967)

Material and Methods

Cell cultures Cultures of a hyperdiploid Ehrlich ascites tumour, denoted ELD, and four of its substrains denoted ELT, SELD clone a, SELD clone f and SELD clone g were used. The isolation of the clones, the culture method in vitro and the characteristics of these strains have been described (HAUSCHKA et coll 1957, LITTBAND 1970, MODIG 1968, REVESZ et coll 1963, 1964). The mean plating efficiency of the different strains varied between 40 and 80 %. The ELT substrains have a duplicated, i.e. hypertetraploid chromosome set in relation to ELD and characterized by an increased radioresistance in in vitro and in vivo conditions (GLAS & REVESZ 1963, LITTBAND 1970, REVESZ & LITTBAND 1964). The cells of SELD clone a, clone f and clone g have chromosome sets in the same ploidy region as ELD but differ in their sensitivity to radiation both in vivo and in vitro (LITTBAND 1970, REVESZ et coll 1963, 1964).

Experimental design Suspension of monodispersed cells were prepared from 6 to 8 day old cultures grown in a monolayer on the surface of stoppered milk dilution bottles. The cells were readily dissociated by treatment with 0.5 % trypsin solution at a temperature of 37° C for 10 minutes. The cells in the number required were explanted in a series of 6 cm pyrex petri dishes containing 4 to 6 ml of nutrient medium after their concentration had been established in a haemocytometer. The culture medium consisted of Eagle's medium in Earle's solution (EAGLE 1959) and was supplemented with 15 % fetal calf serum and antibiotics. The cultures were incubated at 37° C in a moist atmosphere of air with 5 % carbon dioxide added to maintain a pH of 7.0 to 7.2. The cells after 4 days incubation were exposed to radiation and thereafter re-incubated, with a medium change every second day. The number of cells in the population was determined after periods varying from 3 to 21 days. The cells were brought into suspension by exposure to trypsin and by scraping the surface of the dishes with an edged teflon rod. They were counted by a Coulter Model B electronic particle counter. The mean value from duplicate dishes was calculated routinely.

Radiation exposure and control of atmosphere The exposure to roentgen radiation was at a temperature of about 27° C in an air tight plastic chamber of 2 l volumes, with two orifices of 6 mm diameter for the inlet and outlet of

gases The lids of the culture dishes were removed and the nutrient medium withdrawn so that not more than 1 ml fluid was left to cover the cells The chamber was filled with either oxygen or argon with 1.75 % carbon dioxide at a flow rate adjusted to 6 l/min The gases passed through a pre-equilibrated water column warmed to a temperature of 38° C Argon with an oxygen contamination of less than 3 ppm was used and was led to the cells through a system of stainless steel and glass tubes with joints sealed impermeably to oxygen from the atmosphere The outflowing gas passed a water lock and was routinely monitored by an Elcoflux oxygen analyser (Dr Thuedig & Co AG Berlin) The flushing period of the chamber with the respective gases was 11 minutes when a dose of 1 100 R in argon or 450 R in oxygen was delivered or when these doses were given in two fractions When irradiation was made with 2 500 R in argon or 1 000 R in oxygen or with two fractions of these doses the total flushing period was 17 minutes Irradiation was always adjusted to terminate at the end of the flushing period As has been reported elsewhere (LITTBAND 1970) a 4 minute flushing was found to be sufficient for equilibration between the fluid and the gas phase under the conditions described

The dishes after exposure to radiation were refilled with 5 ml fresh medium before incubation Two exposures were made at 4 or 18-hour time intervals as described in the experiments with split doses the cells were incubated aerobically during the intervals between the doses The cells that were irradiated with the total dose at the first exposure were sham irradiated at a time corresponding to the second exposure of the split dose treated cells Unirradiated control cultures were also sham irradiated under the same conditions as the irradiated cells

Radiation factors Radiation was generated in a Siemens roentgen unit at 190 kV and 15 mA with 1.5 mm Al inherent filtration and 1.0 mm Al additional filter half value layer 0.95 mm Cu The dose rate was 235 R/min at the bottom of the culture dishes which were always placed at a distance of 43 cm from the focus The dose delivered was measured by a Philips integrating dosimeter In all the tests the cells when irradiated were attached to the glass surface The correction factor for backscatter as determined from survival measurements with Chinese hamster cells while attached to glass versus plastic, was 1.35 ± 0.05

Results

The population growth of ELD was studied after exposure to roentgen radiation either in a relatively low dose range (450 R delivered in oxygen or

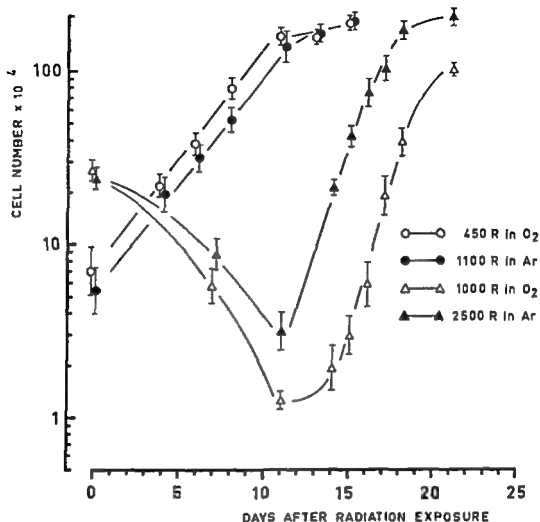


Fig. 1. Growth of F1D ascites tumour cell cultures after irradiation in argon or oxygen. Mean \pm S.E. of the cell number is indicated.

1 100 R in argon) or in a large dose range (1 000 R in oxygen or 2 500 R in argon). Treatment was made either with the total dose at a single exposure or with the dose split into two fractions delivered 4 or 18 hours apart.

Cell populations that originated from an inoculum of 0.3×10^4 cells and reached a size of about 8×10^4 cells after 4 days of growth were irradiated with the doses in the lower range. For exposures with the doses in the higher range populations were used that originated from an inoculum of 10^4 cells and contained about 25×10^4 cells after 4 days of proliferation when the irradiation was administered. As the plating efficiency of ELD in these experiments was $80 \pm 4\%$ (mean \pm S.E.), it can be calculated that the mean doubling time

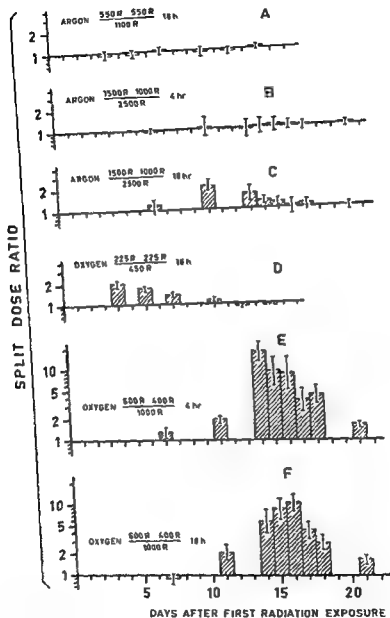


Fig. 2 Split-dose ratios of ELD cells defined as the ratios between the population size at corresponding times after treatment with a single dose and the same dose delivered in two fractions. The irradiation was under either oxic or anoxic conditions. The split dose interval was 4 or 18 hours during which period the cells were incubated aerobically. Columns indicate the mean \pm SE of the split dose ratios.

of the population before irradiation was about 20.5 hours. The value varied within the range of 18–24 hours in the individual experiments.

The mean population sizes at different times after single radiation exposures are given in Fig. 1. When the doses in the low range were given in a series of 10 repeat oxic and in the same number of anoxic experiments, the cell population increased exponentially until it reached a mean number of about 10^6 cells. The mean population doubling time during this growth phase was about 56 hours. The growth then rapidly decelerated and the population approached a maximum level of 2×10^6 cells.

The mean population size decreased gradually in an initial phase when in 13 repeat experiments in argon and in 16 experiments in oxygen the cell populations were exposed to the doses in the high range. Eleven days after treatment in argon and fourteen days after treatment in oxygen the populations started to increase exponentially with a doubling time of 22 hours, i.e. with a doubling time similar to that of the cells before irradiation. After the exponential phase, the growth decelerated and the cell number approached a value of about 2×10^6 .

Split dose ratios defined as the ratio between the population size at corresponding times after treatment with a single dose and the same dose delivered in two fractions are illustrated in Fig. 2. In the case of anoxic irradiation in the low dose range and an 18 hour interval between the split doses (Fig. 2 A), the ratios are close to unity. Similar results were obtained when split doses in the larger range were given anoxically 4 hours apart (Fig. 2 B). Ratios higher than unity were calculated with larger split doses under anoxic conditions and a time interval of 18 hours (Fig. 2 C).

The split dose ratios obtained with irradiations under oxic conditions were significantly larger than unity. Either split doses in the lower range, 18 hours apart, or in the larger range, 4 or 18 hours apart, were delivered. In the former case, the ratios had a maximal value of 2 (Fig. 2 D) and in the latter cases (Fig. 2 E, F) the ratios had a maximal value of about 20.

The relative radiosensitivity of FLD and its four substrains (ELT and SELD, clones a, f and g) was studied in repeat experiments by radiation exposure of populations of each strain when they reached a size of $6\text{--}10 \times 10^4$ cells four days after explantation of an inoculum containing 0.3×10^4 cells. Growth inhibition of the population was taken as the criterion of sensitivity, as indicated by the ratio between the total cell number of the sham irradiated and the irradiated cell population at corresponding times determined in the same experiment. The doubling time of the sham irradiated substrains was within the same range as the parental strain. Exposure doses of 450 R delivered in oxygen or 1100 R in argon were always employed. The results obtained in 5

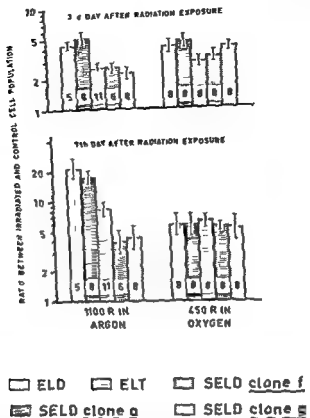


Fig 3 Growth inhibition of ELD and its four substrains as indicated by the ratio between the total cell number of sham irradiated and irradiated populations at corresponding times. Doses of 1100 R in argon or 450 R in oxygen were delivered. Columns show mean \pm SE calculated from repeated experiments in numbers indicated by the figures inside the columns.

to 11 repeat experiments 3 and 7 days after irradiation are illustrated in Fig 3. The growth of ELD and clone a is inhibited by irradiation in argon to a greater degree than the other cell strains. As calculated by covariance and t test analysis, the differences are significant in all cases ($p < 0.01$ or < 0.05). In contrast, irradiation in oxygen inhibits the growth of all the cell strains to a similar extent and differences are statistically insignificant.

Discussion

The cell population at different times after irradiation may be regarded as being composed of cells which either retained reproductive integrity or lost their capacity for unlimited reproduction after suffering lethal damage. We

attempted to calculate the proportion of the two types of cells in the populations presented in Fig 1 by estimating (1) the fraction of cells that retained reproductive integrity, (2) the growth rate of these cells and (3) their division delay

Analysis of the growth curves after oxie irradiation After an exposure dose of 1 000 R, clonogenic cells can be expected to survive in a proportion 4×10^{-4} to 10^{-4} if it is assumed that the survival curve of these cells has an extrapolation number between 2 and 5 and a $D_0 = 91$ R (REVESZ & LITTBAND 1964, and 1969) Accordingly, only about 10 to 25 cells can be expected to retain reproductive integrity in the population irradiated with 1 000 R in our tests Lethally damaged cells may therefore be considered to constitute the great majority of the population for several days after irradiation The decrease of the population, as seen in Fig 1, reflects obviously the rate of disintegration of these cells The finding that only 5 % of the original population was left 11 days after irradiation is in agreement with the observation by TOLMACH (1961) on HeLa cells exposed to a comparable dose

The exponential increase of the population starting 14 days after irradiation is conceivably due to the growth of survivors that retained reproductive integrity During the exponential growth, the population has an average doubling time of 22 hours, which differs slightly from the doubling time of 20.5 hours of the unirradiated cells By extrapolating the exponential part of the growth curve to the day of irradiation, and assuming a division delay of 1 min/rad (ELKIND & WHITMORF 1967, SACHER 1968) corresponding to 23 hours, it can be calculated that on an average a single cell is left in the irradiated population with unaltered growth rate Since the number of clonogenic cells is larger by a decade (cf previous paragraph), it may be concluded that most of the survivors have a considerably lower growth rate This conclusion is in agreement with considerations (DEWEY et coll 1963, NIAS & FOX 1968) according to which the clonogenic cell estimate, arrived at by counting colonies with a minimum of 50 cells, comprises cells that have a prolonged generation time Obviously, such cells can constitute only a negligible fraction of the exponentially growing population 14 days or more after irradiation

When divided doses were delivered, the split dose ratios during the exponential growth phase had a mean value of 8, either 4 or 18 hours being the interval between the doses These split dose ratios are considerably larger than those that have been calculated when the clonogenic capacity was used as criterion of survival and when mean values 2 (plateau phase cells) and 4 (exponentially growing cells) (REVESZ & LITTBAND 1967 and 1969) were found The difference may be interpreted as indicating that repair during the split dose interval

concerns not only radiation damage which affects clonogenic capacity but also damage that leads to a prolongation of the generation time and possibly also to a delay of the division

An extrapolation of the exponential part of the growth curve to the 7th day after irradiation indicated that during the first seven days the great majority of the population consists of lethally damaged cells if the effect of either single or split doses is considered. As indicated in Fig 2 the split-dose ratios calculated at the 7th day do not differ significantly from unity. According to ELKIND *et coll* (1963) cells damaged lethally by a single dose of 1 000 R under aerobic conditions may divide 0.8 times on an average. Due to fractionation of the dose the capacity of such cells for division does not seem to increase or the increase may be too small to be demonstrable by the methods used.

Considering the number of surviving cells and their proliferation rate as being similar to unirradiated cells, it is conceivable that the great majority of the population 11 days after irradiation with a single dose of 1 000 R will still consist of lethally damaged cells. However, after treatment with the same dose divided in two fractions and assuming a mean split dose ratio of 8, a great portion of the population may already by the 11th day consist of rapidly proliferating cells. The split dose ratio of about 2 found by this time (cf Fig 2) may therefore be attributed to the recovery of sublethally damaged cells rather than an increase in the capacity of the lethally damaged cells for division.

After an exposure of 450 R the proportion of the clonogenic cells may be estimated to be about 1.8 to 4.5 %. Accordingly, about 1.2 to 3×10^3 cells may be expected to retain reproductive integrity in the original population. The observation that the growth curve reached plateau level after 11 days of cell proliferation and before or on the 11th day may help to estimate the number of survivors that have a similar generation time as unirradiated cells. Assuming a division delay of 1 min/rad and considering a doubling time of 20.5 hours, 11 may be calculated that the number of surviving cells with an unaltered proliferation rate is in the range 0.4 — 2.6×10^3 immediately after irradiation. This range is lower than that of the number of clonogenic cells as can be estimated from the survival curves. As discussed above the difference is conceivably due to the counting of clones with a minimum of 50 cells in the clonogenic test, i.e. including survivors with prolonged generation times. In view of this approximation of the survival ranges it may be concluded that most of the population (80 to 100 %) four days after irradiation consists of cells that have lost reproductive integrity. No safe estimate of the proportion of such cells can be made during the subsequent phases of the growth curve. The lethally damaged cells will constitute a gradually decreasing portion of the total population due to their disintegration and the simultaneous proliferation

of surviving cells. The average doubling time of 56 hours of the population may therefore be considered to be due to the composite kinetics of cell gain and cell loss.

Since surviving cells constitute a minor fraction of the population during the first four days after irradiation, the increase in the population during this period may be attributed to the proliferation of the lethally damaged cells. It may be calculated from the population increase by a factor of 3.1 that these cells are capable to complete, on an average, a 1.6 division. During the subsequent growth period, they may be capable of further divisions, the number of which cannot however be estimated from the data with any confidence. The value 1.6 is in reasonable agreement with the observation by ELKIND *et coll.* (1963) when Chinese hamster cells were exposed aerobically to a comparable dose of 600 rad.

Also when the 450 R was delivered in two fractions, the population reached plateau level during the period between 8 and 11 days after treatment. The number of clonogenic cells with a growth rate similar to the unirradiated cells can be assumed therefore to be also within the range $0.4-2.6 \times 10^3$, as discussed above. Consequently, also in this case the lethally damaged cells will constitute the great majority of the population (90 to 100 %) during the first four days following irradiation. By the fourth day their number increased by a factor of 6.2. This indicates that on an average 2.6 cell divisions occurred. In comparing this number with the number of divisions after a single exposure to the same total dose, it may be concluded that, due to fractionation, an additional division took place. Repopulation during the fractionation interval by a factor of 2 can be excluded as an explanation in view of the result of complementary experiments performed to test this possibility. In those experiments the size of a population originating from 0.3×10^4 cells was determined, on the one hand, after four days of growth and, on the other hand, after four days of growth followed byoxic irradiation with 225 R and an additional interval of 18 hours. The mean ratio between the two population sizes, as determined in 10 repeat experiments, was 0.97 ± 0.16 . This indicates that the population size does not change during the time interval between the split doses used in the experiments.

This result is in agreement with what can be expected when considering the following points:

1. The doubling time of the cells extends the time interval between the split doses even without taking division delay into account.

2. Repopulation, if it should occur, would increase the fraction of clonogenic survivors and consequently the growth curve could be expected to reach the plateau phase earlier than when the single total dose is given. This was however not the case, as indicated by the data in Fig. 2 B.

3 The split dose ratios after *oxic* irradiation with a total dose of 1 000 R are similar when either 4 or 18 hours separated the doses (cf Fig 2 E F) if repopulation occurred, the ratios after the latter interval ought to be increased

4 When irradiation is performed under *anoxic* conditions with 1 100 R which reduces the number of survivors to a similar level as *oxic* irradiation with 450 R the population size after treatment with split doses separated by an interval of 18 hours is not increased in comparison to the population size after a single treatment with the same total dose obviously repopulation would have increased the size (cf discussion below and Fig 2 A)

Thus since repopulation during the fractionation interval may be excluded as an explanation a certain repair of the radiation damage of the lethally injured cells may be considered to account for the increase of the population treated with split doses The repair would increase the capacity of the lethally damaged cells for limited reproduction permitting the cells to complete an additional division This interpretation implies that the repair of the radiation damage which results in an increased reproductive capacity of cells may proceed not only in sublethally but also in lethally injured cells In confirmation of the suggestion by ELKIND *et coll* (1967) it can be concluded that lethal and sublethal cellular damage may be qualitatively similar and may differ from each other only in degree In considering the finding that no repair of the reproductive capacity of sublethally irradiated cells was demonstrable when a total exposure dose of 1 000 R was delivered it would seem that the repair mechanism which concerns this damage may itself be radiosensitive

Anoxic growth curves and the oxygen enhancement of the radiation effect

The growth curve of the cell population after the exposure dose of 1 100 R in argon is similar to the growth curve obtained when the exposure dose was 450 R in oxygen (cf Fig 1) This indicates that the proliferation of the lethally damaged cells during an initial period after irradiation when they constitute most of the population, is affected to the same extent in both cases and consequently the oxygen has a dose modifying effect indicated by the ratio between the doses i.e. a factor 2.44 Since the growth curves of the *oxically* and *anoxically* irradiated populations reach plateau level at about the same time after irradiation it may be concluded furthermore that oxygen has a similar dose modifying effect with regard to the number of the survivors that retain reproductive integrity as with regard to survivors that preserve an unaltered growth rate

When the growth curve after 2 500 R delivered in argon is compared to the growth curve after 1 000 R delivered in oxygen it is apparent that the exponential phase in the former instance starts about three days earlier By

extrapolation of the exponential curve to the time of irradiation, it may be calculated that ~ 10 cells may have survived the anoxic treatment with reproductive integrity and unaltered growth rate. Considering the anoxic survival curve of these cells with a D_0 235 R and an extrapolation number 1, the number of clonogenic survivors may be estimated to be about the same.

The difference between the growth curves when irradiation is administered with 2500 R and 1000 R under anoxic and oxic conditions, respectively, indicates that the dose modifying effect of oxygen is expressed by a larger enhancement factor than the ratio between the doses delivered, i.e. 2.5. From the estimate of the surviving fractions, arrived at by extrapolation of the exponential part of the growth curves, a value of about 3 may be calculated as an approximate factor for the oxygen enhancement. This value is larger in comparison to the value that was calculated when the higher survival level was considered (cf. previous paragraph). A smaller oxygen enhancement factor, corresponding to a larger survival level, is in agreement with the previous observation in the experiments in which clonogenic capacity was the criterion of the radiation effect, and in which the corresponding enhancement factors may be estimated to be 2.2 and 2.5 (REVEZ & LITTEBRAND 1964). The dependency of the enhancement factors upon the survival level may be regarded as a consequence of the difference between the extrapolation number of oxic and anoxic radiation survival curves, as discussed previously.

Apparently the enhancement factors calculated from the data of the growth curves are larger than the corresponding factors arrived at from the data of the clonogenic survival curves. If further experiments prove the significance of those differences, a possible explanation may be a differential oxygen enhancement of the radiation damage that concerns retardation of the growth and the damage that concerns clonogenic capacity. In order to test this possibility in current experiments the differences between the colony size distribution of oxidically and anoxically irradiated clonogenic cell populations are being studied.

When radiation exposures were made under anoxic conditions, the split dose ratios were either close to unity (cf. Fig. 2-A and 2-B) or varied between 1 and 2 (cf. Fig. 2-C). Previous experiments have indicated that the clonogenic capacity of the cells fails to recover when oxygen is absent during irradiation (LITTEBRAND, 1970, LITTEBRAND & REVEZ 1969). As discussed above little repopulation can occur during the particular fractionation intervals after the doses employed. The increased split dose ratios indicated in Fig. 2-C may therefore be interpreted as indicating a certain repair which conceivably concerns the damage resulting in growth retardation and, possibly, division delay. A similar interpretation may be given also to the increased split dose ratios found after oxic irradiation (cf. Fig. 2-E and 2-F). If this interpretation is correct it may

be concluded that the recovery of the damage leading to growth retardation and division delay is less oxygen dependent than the recovery of the clonogenic capacity. In view of the standard error of the determination indicated in Fig. 2 A and 2 B such a recovery in the growth retardation cannot be excluded completely either in these anoxic experiments.

Sensitivity differences between cellular substrains In view of the fact that ELD and its four substrains (ELT, SELD clones a, f and g) used in the present experiments have a similar growth rate, the data presented in Fig. 3 indicate that sensitivity differences do exist between the strains when growth inhibition is the criterion of sensitivity. ELT clones f and g appear to be more resistant to anoxic radiation than the parental line or clone a. As discussed above, it may be assumed that the overwhelming majority of the cell population three days after exposure to 1100 R under anoxic conditions consists of cells that suffered lethal radiation damage. The difference in the ratio between the size of the irradiated and control cell population during the first three days is thus due to a difference in the proliferative capacity of the lethally damaged cells. It may be calculated that while lethally damaged ELD and clone a cells completed on an average 1.6 and 1.0 divisions respectively, the ELT clones f and g cells divided about 2.3 times.

Seven days after the anoxic irradiation, the populations consist conceivably of mixed lethally damaged and proliferating surviving cells. In comparison to ELD, the populations of ELT clones f and g cells are larger by a factor of 2.4, 4.7 and 4.5 respectively. The larger population sizes may now be interpreted as being due partly to the increased proliferative capacity of the lethally damaged cells and partly to the greater number of clonogenic survivors. From the data obtained in a previous investigation in which the clonogenic survival of ELT and clone g cells were studied, it may be calculated that a population 2.3 and 2.8 times larger than ELD respectively can be expected to survive after anoxic exposure to 1100 R (REVEZ & LITTBAND 1967; LITTBAND 1970); the c values are in a good agreement with the corresponding values indicated in Fig. 3. The observation that the population size of clone a does not differ significantly from ELD after anoxic irradiation is also in agreement with the earlier finding that the clonogenic survival of these two strains is similar.

As previously mentioned, the sensitivity differences can be associated with the difference in ploidy in the case of ELT, which has a duplicated chromosome set, and the difference in the content of intrinsic acid soluble sulphhydryl compounds in the case of clones f and g, in which the concentration is larger by a factor of about 1.3 relative to ELD (LITTBAND 1970). Clone a has the same concentration of sulphhydryl groups and the same chromosome number as ELD.

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The difference between the growth curves when irradiation is administered with 2 500 R and 1 000 R under anoxic and oxic conditions, respectively, indicates that the dose modifying effect of oxygen is expressed by a larger enhancement factor than the ratio between the doses delivered i.e. 2.5. From the estimate of the surviving fractions, arrived at by extrapolation of the exponential part of the growth curves, a value of about 3 may be calculated as an approximate factor for the oxygen enhancement. This value is larger in comparison to the value that was calculated when the higher survival level was considered (cf previous paragraph). A smaller oxygen enhancement factor, corresponding to a larger survival level, is in agreement with the previous observation in the experiments in which clonogenic capacity was the criterion of the radiation effect, and in which the corresponding enhancement factors may be estimated to be 2.2 and 2.5 (REVEZ & LITTBRAND 1964). The dependency of the enhancement factors upon the survival level may be regarded as a consequence of the difference between the extrapolation number of oxic and anoxic radiation survival curves, as discussed previously.

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SUMMARY

An investigation was performed with cultures of the ELD ascites tumour to obtain information on the multiplication of cells irradiated with a single or a split dose of radiation in the presence or absence of oxygen. Oxygen enhanced the radiation damage concerned with clonogenic capacity to a greater extent than the damage concerned with growth retardation. Recovery due to fractionation was observed for both types of damage. The reaction of the cell strains appears to be related to differences in their ploidy grade and the content of inherent nonprotein bound sulphhydryl groups.

ZUSAMMENFASSUNG

Kulturen des ELD Aszestumors wurden benutzt um die Zellenvermehrung nach Bestrahlung mit einer Einzeldosis oder mit einer unterteilten Dosis mit oder ohne Sauerstoffzufuhr zu studieren. Sauerstoff erhöhte die Strahlenschädigung hinsichtlich der klonbildenden Kapazität in höherem Ausmass als hinsichtlich der Wachstumshemmung. Erholung als Folge von Fraktionierung war für beide Typen von Schädigung zu beobachten. Die Reaktion verschiedener Zellkulturen scheint von Unterschieden in ihrem Ploidygrade und deren Gehalt an nicht proteingebundenen Sulphydrylgruppen abzuhängen.

RÉSUMÉ

L'auteur a fait des recherches sur des cultures de tumeur d'ascite ELD pour étudier la multiplication des cellules irradiées par une dose unique de radiation ou par une dose fractionnée en la présence et en l'absence d'oxygène. Les radio-lésions qui ont un effet sur l'aptitude clonogénique sont plus augmentées par l'oxygénation que les lésions qui interviennent dans le retard de croissance de la tumeur. Dans ces deux types de lésions on a observé une restauration due au fractionnement. La réaction des souches de cellules paraît liée à des différences dans leur degré de ploïdie et dans la teneur en groupes sulphydryle inhérents non liés aux protéines.

REFERENCES

- DEWEY W. C., HUMPHREY R. M. and CORK A. Comparison of cell multiplication and colony formation as criterion for radiation damage in cells grown *in vitro*. *Int J Radiat Biol* 6 (1963) 463.
- EAGLE H. Amino acid metabolism in mammalian cell cultures. *Science* 130 (1959) 432.
- ELKIND M. M. and WHITMORE G. F. *The radiobiology of cultured mammalian cells*. Gordon and Breach, New York, London and Paris 1967.
- HAINY A. and VOLZ K. W. Radiation response of mammalian cells grown in culture. IV. Dose dependence of division delay and postirradiation growth of surviving and nonsurviving Chinese hamster cells. *J. nat. Cancer Inst.* 30 (1963) 705.
- SUTTON GILBERT H., MOSES W. H. and KAMPER C. Sub-lethal and lethal radiation damage. *Nature (Lond.)* 214 (1967) 1088.
- GLAS U. and RÉVÉSZ L. Radiosensitivity of selected sub lines of a tumour-cell population tested in the presence or absence of oxygen. *Int J Radiat Biol* 6 (1963) 69.

The observation that sensitivity differences, apparent after radiation exposure under anoxic conditions, disappear when exposure is made in the presence of oxygen, is in agreement with earlier findings obtained in different experimental systems (REVEZS *et coll* 1963). The possible mechanisms underlying this effect of oxygen have been discussed in a previous publication (LITTBRAND 1970).

Conclusions

The multiplication of ELD ascites tumour cells and four of its substrains were studied after exposure to single or split doses of roentgen radiation under oxic or anoxic conditions. The cell population increased exponentially with a doubling time of 56 hours after exposure doses of 450 R in oxygen and 1 100 R in argon. When doses of 1 000 R in oxygen and 2 500 R in argon were delivered, the cell population decreased during an initial period of 11 days after treatment and, subsequently, grew exponentially with a doubling time of 22 hours. The ratio between the size of the cell populations irradiated with a single dose, and with the same dose split into two fractions, was between 1 and 2 in cases of anoxic irradiation exposures, and between 2 and 20 in cases of oxic exposures. After oxic irradiation, the growth of the different cell strains was inhibited to a similar extent. On the other hand, after anoxic irradiation, the cell strains presented characteristic differences in their reaction to the growth inhibitory effect of radiation.

The growth curves were analysed in view of the clonogenic survival of the cells after comparable exposure doses. This analysis indicates that a great proportion of clonogenic survivors have a reduced growth rate, in addition to the clonogenic capacity recovery during the split dose interval concerns the growth rate and possibly also division delay. The capacity of lethally injured cells for a limited number of divisions is improved by split dose treatment, repair of radiation damage leading to recovery is, to a great extent, dependent upon the presence of oxygen during irradiation.

The particular reaction of different cell strains can be related to the differences in their ploidy grade and the content of inherent, nonprotein bound sulphhydryl groups.

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The author wishes to thank Dr L. Revesz for his valuable suggestions and criticism and Miss Inga Birgen, Mrs Ingegerd Hedlof and Miss Rut Jonsson for their technical assistance. The work was supported by grants from the Swedish Cancer Society, The Sir Samuel Scott of Jews Trust and The Swedish Medical Society.

LONG TERM CYTOGENETIC AND CLINICAL CONTROL OF A CHILD FOLLOWING INTRAUTERINE IRRADIATION

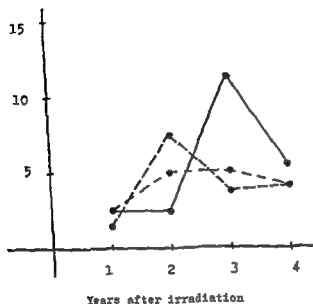
by

MARIA KUČEROVÁ

Morphologic and cytogenetic lesions of the foetus are presumed to occur in irradiated pregnant women especially with radiation in the therapeutic range. Experimental studies in lower mammals (HICKS 1953, SOUKUP *et coll* 1965) and the examination of irradiated human foetuses (HORBS 1950, MILLER 1956, DRISCOLL *et coll* 1963, KLAUBER 1965, DEKABAN 1968) confirm this presumption from the morphologic point of view but relatively few chromosome studies in irradiated human foetuses have so far been performed. SATO (1966), MACEN *et coll* (1967) analysed the chromosomes from fibroblasts of dead foetuses in long term cultures. Chromosome aberrations in lymphocytes from living subjects after intrauterine irradiation with a diagnostic dose were reported by LEJEUNE *et coll* (1964) and with a therapeutic dose by KUČEROVÁ (1967a). In the last mentioned paper a child surviving after irradiation in the second half of pregnancy was described. This child has now been systematically observed for 4 years, and the findings are summarized in the present paper.

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- HAUSCHKA T S GRINNELL S T, RÉVÉSZ L and KLEIN G Quantitative studies on the multiplication of neoplastic cells in vivo IV Influence of doubled chromosome number on the growth rate and final population size J nat Cancer Inst 19 (1957) 13
- LITTBAND B Survival characteristics of mammalian cell lines after single or multiple exposures to roentgen radiation under oxic or anoxic conditions Acta radiol Ther Phys Biol 9 (1970) 257
- and RÉVÉSZ L The effect of oxygen on cellular survival and recovery after radiation Brit J Radiol 42 (1969) 914
- MODIG H Intrinsic sulphhydryl levels and development of cellular resistance to γ irradiation Studia biophys (Berlin) 12 (1968) 189
- NIAS M A and FOX M Minimum clone size for estimating normal reproductive capacity of cultured cells Brit J Radiol 41 (1968) 468
- REVÉSZ L and LITTBAND B Variation of the relative sensitivity of closely related neoplastic cell lines irradiated in culture in the presence or absence of oxygen Nature (Lond) 203 (1964) 742
- — Qualitative differences between cellular radiation damage suffered under oxic and anoxic conditions The dual effect of oxygen Proc International Conference on Radiation biology and Cancer Kyoto 1966 p 67 Radiation Society of Japan 1967
- — Culture age and cellular radiosensitivity Exp Cell Res 55 (1969) 283
- GLAS U and HILDING G Relationship between chromosome number and radiosensitivity of tumour cells Nature (Lond) 198 (1963) 260
- SACHIER G A Mathematical analysis of the division delay produced by ionizing radiations Radiat Res 33 (1968) 644
- TOLMACH L J Growth patterns in γ irradiated HeLa cells Ann N Y Acad Sci 95 (1961), 743

Per cent
of cells

Percentages of B (— — —) C_H (— · — · —) and C_A (————) cells of the child during four years after irradiation

In the diagram accompanying this paper the cells have been classified according to the Edinburgh group of authors (BUCKTON et coll 1962) as B, C_H and C_A. This classification has been used by many authors; the present results can thus be compared with those of others.

Four examinations in all were performed under standard conditions in the child (in December 1965, 1966, 1967 and 1968). In 1965 and 1966 peripheral blood from the mother was also examined.

Results

Detailed cytogenetic analysis indicated a significant increase of mitoses with chromosome aberrations in all blood samples from child and mother. The tabulated results (Table 1) disclosed that the percentage of aberrant mitoses in the child's blood increased with post irradiation from 6% in the first year to over 15% in the second year and up to 20% in the third year of life. The percentage fell slightly to 13.6% in the fourth year. It is still not certain whether the percentage will further decrease or not. A sharp increase in the percentage of aberrations

At the same time and for two years chromosomes of the peripheral blood of the mother were analysed. It was of interest to find which types of aberrations occurred in the child and the mother, and the time course of these changes.

Case report

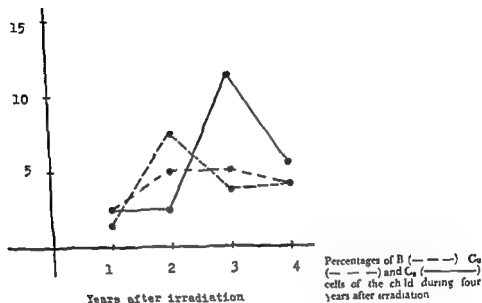
The father was 39 at the time of the child's birth and in good health. Two elder sisters (one a step sister) were normal in body and mental development. Carcinoma of the cervix uteri was discovered in the mother during the third pregnancy when she was 36 years old. She was therefore given roentgen irradiation in the course of the 5th and 6th months of pregnancy. The radiation factors were 180 kV, 10 mA, filter 1 mm Cu. Four fields all 10 cm × 15 cm were exposed to radiation: anterior left, anterior right, posterior left and posterior right with 27.6 R/min. individual exposures 270 R. total surface exposures of the individual fields 3580 R. The total radiation dose was divided into 26 daily radiations. The dosage received by the child is not known.

Hysterectomy with Caesarean section was performed in the 8th month of pregnancy. The mother died of generalized carcinoma 2 years after delivery. The birth weight of the child was 1960 g and its length 44 cm. No congenital malformations were observed. The child sat at 9 months, stood at 12 months, walked at 21 months and began to talk at 36 months. At the age of 1 year our patient had febrile convulsions, later followed by epileptic seizures of the type major epilepsy.

At the time of this report the child was 4 1/2 years old with evidence of microcephaly. Her height was 95 against 103.9 cm, weight 11.5 against 16.5 kg, the head circumference 46 against 51 cm and that of the breast 49 against 55 cm. The mental development of the child corresponded to that of a 2 year old and of a low grade imbecile. She was passive and apathic. Comprehension and articulation of speech were poor. Neurologic examination suggested slight cerebellar changes and EEG has been abnormal with manifestations of asymmetry. Slight anaemia and leucocytosis have been repeatedly observed.

Methods of investigation: A standard cytogenetic method of short term peripheral blood cultures, a modification of Moorhead's method (MOORHEAD et coll. 1960), was employed. The cultures were grown in Epl medium (USOL-Institute of Sera and Vaccines, Praha) and calf serum 25 % for an average of 64 hours, and stained with Giemsa. All mitoses were photographed, cut and analysed and only those treated in this manner are described in the results (to avoid monotrismy being overlooked).

In the classification of cells, the mitoses are grouped as hypomodal, modal, hypermodal and polyploid, according to the chromosome number, and as normal and aberrant mitoses according to structural changes. Aberrant mitoses are divided into simple (resulting from simple breaks) and complicated (arising from multiple breaks and reunions) and monotrismy (cells with chromosomes of normal shape that cannot however be correctly divided into groups of the Denver nomenclature). This has been described in detail in a previous paper (KUČEROVÁ 1967b).

Per cent
of cells

In the diagram accompanying this paper the cells have been classified according to the Edinburgh group of authors (BUCKTON et coll 1962) as B, C α and C β . This classification has been used by many authors; the present results can thus be compared with those of others.

Four examinations in all were performed under standard conditions in the child (in December 1965, 1966, 1967 and 1968). In 1965 and 1966 peripheral blood from the mother was also examined.

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Detailed cytogenetic analysis indicated a significant increase of mitoses with chromosome aberrations in all blood samples from child and mother. The tabulated results (Table 1) disclosed that the percentage of aberrant mitoses in the child's blood increased with post irradiation from 6.5% in the first year to over 15% in the second year and up to 20% in the third year of life. The percentage fell slightly to 13.6% in the fourth year. It is still not certain whether the percentage will further decrease or not. A sharp increase in the percentage of aberrations

Table 1

Chromosome analysis of peripheral leukocytes of child and mother after therapeutic irradiation and in normal controls

Time after irradiation	Number of mitoses			Total	Aberrant mitoses			Types of aberrations			Per cent polyploid cells
	Number of chromosomes				Num ber	Per cent	Total	number/per cent			
	<46	=46	>46					S	C	MT	

Child											
One year	19	173	4	196	13	66	13	$\frac{3}{42}$	$\frac{6}{46}$	$\frac{4}{30}$	11.2
Two years	3	36	1	40	0	150	7	$\frac{4}{57}$	$\frac{2}{29}$	$\frac{1}{14}$	—
Three years	21	51	8	80	16	200	22	$\frac{7}{32}$	$\frac{4}{18}$	$\frac{11}{50}$	1.2
Four years	23	49	1	73	10	136	13	$\frac{4}{31}$	$\frac{5}{58}$	$\frac{4}{31}$	2.6
Mother											
One year	13	67	3	83	4	60	5	$\frac{1}{20}$	$\frac{2}{40}$	$\frac{2}{40}$	5.6
Two years	16	41	2	59	8	135	11	$\frac{7}{63}$	$\frac{2}{18}$	$\frac{2}{18}$	1.6
Controls	95	577	33	725	7	09	7	$\frac{3}{43}$	—	$\frac{4}{57}$	0.6

was also noticeable in the mother, who had 6 % of aberrations in the first post irradiation year and 13.5 % of aberrant mitoses in the second year.

The percentage of polyploids, which was high in the mother and child in the first year, fluctuated in the following years around the usual percentage in the controls.

After the classification of the observed aberrations into three basic categories (simple, complicated and monotrisonic), certain fluctuations were noted during the following four years but the direction of these changes was not uniform. The most recent results (i.e. 4 years after irradiation) indicate that all three categories of aberrations were more or less uniformly represented.

A still more detailed analysis of aberrant mitoses i.e. the study of individual aberrations but not of groups of aberrant mitoses (see Table 2) revealed a gradual decline in the incidence of dicentric chromosomes (from 2.5 % to zero).

Table 2

Incidence of individual aberrations at different intervals after irradiation

Time after irradiation	B	B per cent	F	AC	DC	DC per cent	RC	TR	M	MT	MT per cent	Number of all aberrations	Number of all analysed mitoses	Number of abnormal mitoses
Child														
One year	3	15			5	25		1		4	2	13	196	13
Two years	3	75		1	1	25		1		1	25	7	40	6
Three years	4	50		3	1	12	1		2	11	135	22	80	16
Four years	3	41	1	3				1	1	4	54	13	73	10
Mother														
One year	2	24			2	24				1	12	5	83	5
Two years	3	50		4	1	17		1		2	35	11	59	8
Control	3	04								4	05	7	795	7

B = break F = fragment AC = acentric chromosome DC = dicentric chromosome RC = ring chromosome TR = translocation M = marker MT = monotrismy

and an increase in the monotrismy (from 2% up to 54%). Both changes can be anticipated because the dicentric chromosomes are considered to be an unstable type of aberration and monotrismy a stable one. The increase in the number of breaks (from 15 to 41%) is interesting but difficult to explain. The possibility cannot be excluded that this could be partly due to the technical procedure used which hardly affects the incidence of the other aberrations however. After classifying the aberrant mitoses observed in this case as B, C and C cells unexpected results were obtained. The number of C cells involving aberrations regarded as unstable, usually declined following a rapid increase after irradiation. In our material the level of these cells was practically

unchanged during the 4-year observation period and remained slightly elevated, with small fluctuations.

The C₂ cells, involving aberrations regarded as stable usually slightly increase in number after irradiation, then slowly decline and remain at a somewhat increased level for several years. We found that their numbers fluctuated significantly, were relatively stable and increased slightly between the first and second years, increased much between the second and third years, and then decreased rapidly between the third and fourth years to a level higher than in the second and third years.

Cells B, not included in the post irradiation changes by the Edinburgh group (they may be artificial in origin and their number ought to be relatively stable), exhibited significant fluctuations in our studies. It is interesting that the number of B cells increased as the number of mitoses in the culture decreased. As the same blood volume (10 ml) was always used, and all suitable mitoses were analysed, an explanation may be that the less readily the cells multiplied in the culture, the less resistant were their chromosomes against mechanical damage.

The results of chromosome analysis in the mother and child could be compared only in the first and second years after irradiation. The percentage of aberrant mitoses and the proportion of different types of aberrations did not differ substantially during the first and second investigations. Differences could be found in following the percentage of individual aberrations and polyploidy. The percentage of dicentric chromosomes in the mother decreased (from 2.4 to 1.7 %) between the first and second year and in the child between the third and fourth year. Monotrisomy increased more significantly in the mother than in the child, from 1.2 to 3.5 % in the mother and from 2 to 2.5 % in the child. The percentage of polyploidy, which was significantly increased in both the mother and child in the first year after irradiation (5.6 % in the mother and 11.2 % in the child), was in the child double that observed in the mother.

Discussion

The results of the cytogenetic investigation revealed an unexpected increase in the proportion of aberrant mitoses during the first three years following irradiation and are in variance with our previous findings in adult females irradiated with a roentgen therapeutic dose (KUČEROVÁ, *in press*) and also with findings of other authors (BUCKTON *et coll.* 1962, BENDER & GOOCH 1963, MILLARD 1965, BUCKTON *et coll.* 1967), who have reported that the total number of chromosome aberrations in peripheral blood of irradiated subjects always decreases with a prolonged time interval after irradiation.

The possibility is not excluded that the exceptional circumstances under which

the chromosome aberrations originated in the present case that is, the period of intrauterine life, could explain the unusual increase in the aberrations. This increase was however also noted in the mother between the first and second year following irradiation. The progressive generalization of the malignancy might have had some bearing.

Unfortunately there are still too many gaps in our information on the behaviour of lymphocytes with chromosome aberrations of all types in the human body. The mechanism controlling their level is quite unknown. The variations in the number of cells with aberrations considered as stable and those with aberrations regarded as unstable cannot as yet be satisfactorily explained.

The approach of other authors who analysed the chromosomes in foetuses after intrauterine irradiation differed somewhat from that used in our case. The radiation effects in the initial months of pregnancy on embryonic cells in long term tissue cultures were described by SATO (1966) and MACEK *et coll.* (1967). LEJEUNE (1964) reported chromosomal changes in the peripheral blood and skin of a child irradiated with a diagnostic dose in the first half of the first month of pregnancy. The child exhibited some malformation as well as mental retardation and certain chromosome aberrations (surplus chromosomes). According to DEKABAN (1968) it is not certain whether during the first two weeks of pregnancy the foetus can be damaged by irradiation.

The clinical lesions in the present case correspond rather closely to lesions produced by experimental irradiation in young mice and rats (HICKS 1953, HULSE 1964). Those irradiated in the second half of gestation presented evidence of retardation in growth and general hypoplasia, diminished volume of the brain, decreased thickness of the cortex cerebri and changes in the EEG as well as disturbances in locomotor coordination. Models of oligophrenia could be experimentally induced. HICK reports in addition to lesions of the cortex cerebri changes in the cerebellum in young rats irradiated in the last trimester of gestation.

All the above disturbances including slight cerebellar lesions were observed in the child described in this study. The basic disturbances such as microcephaly as well as growth and psychomotor retardation fully agree with clinical findings in other children after intrauterine irradiation with therapeutic doses as reported by DEKABAN (1968). They also concur with those in children after intrauterine irradiation in Hiroshima as given by MILLER (1956) and PLUMER (1952). However epileptic seizures have not yet been reported in the cases described in the literature. The clinical signs observed in the child examined correspond to irradiation performed between the 16th and 20th weeks of pregnancy according to DEKABAN's table though the patient was actually irradiated between the 20th to the 24th weeks when to cite DEKABAN, no anomalies should arise. It seems

that the time limits of the radiation effects have not been determined accurately enough, they may alter to a certain extent, or overlap. The situation may nevertheless be affected by varying individual influences.

HOBBS (1950) and KLAUBER (1965) described cases of intrauterine irradiation of the child in the second half of pregnancy entirely free from any clinical abnormality. Presumably irradiation was performed after the critical period described by DEBABAN.

Acknowledgement

The technical assistance of Miss Olga Tilschová is gratefully acknowledged.

SUMMARY

The results of a 1 year cytogenetic and clinical control of a child subjected in the fifth and sixth months of pregnancy to a therapeutic roentgen dose for carcinoma of the cervix uteri in the mother are reported. A detailed analysis of chromosome aberrations produced some atypical findings which are discussed. The mother also underwent cytogenetic investigations.

ZUSAMMENFASSUNG

Die Resultate einer vierjährigen cytogenetischen und klinischen Kontrolle eines Kindes werden vorgelegt. Die Mutter wurde wegen eines Cervixcarcinoms während den fünften und sechsten Monaten der Schwangerschaft mit einer therapeutischen Strahlendosis behandelt. Eine detaillierte Analyse der Chromosomenaberrationen führte zu atypischen Befunden die diskutiert werden. Die Mutter wurde auch cytogenetisch untersucht.

RÉSUMÉ

L'auteur présente le résultat de l'étude cytogénétique et clinique poursuivie pendant quatre ans sur un enfant soumis au cours des cinquième et sixième mois de la grossesse à une dose thérapeutique de rayons de roentgen pour un cancer du col utérin de sa mère. L'analyse détaillée des aberrations chromosomiques donnait quelques résultats atypiques qui sont examinés. La mère était soumise elle aussi à des examens cytogénétiques.

REFERENCES

- BENDER M. A. and GOOCH P. C. Persistent chromosome aberrations in irradiated human subjects. II. Three and one half year investigation. *Radiat. Res.* 18 (1963) 389.
 BUCKTON K. F., COURT BROWN W. M. and SMITH P. G. Lymphocyte survival in men treated with X rays for ankylosing spondylitis. *Nature* 214 (1967) 470.
 — JACOBS P. A., COURT BROWN W. M. and DOLL R. A study of the chromosome damage persisting after X ray therapy for ankylosing spondylitis. *Lancet* 1962 II p. 676.

- COURT BROWN W M BUCKTON K E and McLEAN A S Quantitative studies of chromosome aberrations in man following acute and chronic exposure to X rays and gamma rays *Lancet* 1965 I p 1239
- DEKABAN A S Abnormalities in children exposed to X radiation during various stages of gestation Tentative time table of radiation injury to the human fetus I *J nucl Med* 9 (1968) 471
- DRISCOLL S G HICKS S P COPENHAYER E II and EASTERDAY C L Acute radiation injury in two human fetus *Amer J Path* 76 (1963) 113
- HICKS S P Developmental malformations produced by radiation *Amer J Roentgenol* 69 (1953) 272
- HOBBS A A Fetal tolerance to roentgen rays *Radiology* 54 (1950) 242
- HULIE E V The effects of ionising radiation in the embryo and foetus A review of experimental data *Clin Radiol* 15 (1964) 312
- KLAUBER W A Pregnancy complicated by carcinoma of the cervix *J Ill C. med Ass* 61 (1965) 340
- KUCEROVA M (a) Chromosome analysis of an infant after intrauterine irradiation *Acta radiol Ther Phys Biol* 6 (1967) 410
- (b) Comparison of radiation effects in vitro upon chromosomes of human subjects *Acta radiol Ther Phys Biol* 6 (1967) 441
- Chromosome aberrations after X ray therapy To be published in *J Génét hum*
- LEJEUNE J BERGER R ARCHAUBAULT L et coll Mosaïque chromosomique probablement radio-induite in utero *C R Acad Sci (Paris)* 259 (1964) 485
- MACEK M UHER J CERNOCH A and GOETZ P Účinek rtg ozáření na chromozomy embryonálních tkání (In Czech) *Cs gynek* 32 (1967) 587
- MILLARD R E Abnormalities of human chromosomes following therapeutic irradiation *Cytogenetics* 4 (1965) 277
- MILLER R W Delayed effects occurring within the first decade after exposure of young individuals to the Hiroshima atomic bomb *Pediatrics* 18 (1956) 1
- MOORHEAD P S DOWELL P C MALLMAN W J et coll Chromosome preparations of leucocytes cultured from human peripheral blood *Exp Cell Res* 20 (1960) 613
- PLUMMER G Anomalies occurring in children exposed to the atomic bombs in Hiroshima *Pediatrics* 10 (1952) 687
- SATO H Chromosomes of irradiated embryos *Lancet* 1966 II p 551
- SOLKUP S W Chromosome change in rat embryos following X irradiation *Cytogenetics* 4 (1965) 130

that the time limits of the radiation effects have not been determined accurately enough, they may alter to a certain extent, or overlap. The situation may nevertheless be affected by varying individual influences.

HOBBS (1950) and KLAUBER (1965) described cases of intrauterine irradiation of the child in the second half of pregnancy entirely free from any clinical abnormality. Presumably irradiation was performed after the critical period described by DEKABAN.

Acknowledgement

The technical assistance of Miss Olga Tilschová is gratefully acknowledged.

SUMMARY

The results of a 4 year cytogenetic and clinical control of a child subjected in the fifth and sixth months of pregnancy to a therapeutic roentgen dose for carcinoma of the cervix uteri in the mother are reported. A detailed analysis of chromosome aberrations produced some atypical findings which are discussed. The mother also underwent cytogenetic investigations.

ZUSAMMENFASSUNG

Die Resultate einer vierjährigen cytogenetischen und klinischen Kontrolle eines Kindes werden vorgelegt. Die Mutter wurde wegen eines Cervixcarcinoms während den fünften und sechsten Monaten der Schwangerschaft mit einer therapeutischen Strahlendosis behandelt. Eine detaillierte Analyse der Chromosomenaberrationen führte zu atypischen Befunden die diskutiert werden. Die Mutter wurde auch cytogenetisch untersucht.

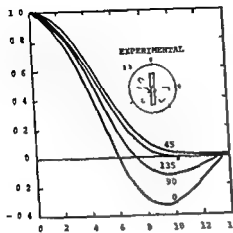
RÉSUMÉ

L'auteur présente le résultat de l'étude cytogénétique et clinique poursuivie pendant quatre ans sur un enfant soumis au cours des cinquième et sixième mois de la grossesse à une dose thérapeutique de rayons de roentgen pour un cancer du col utérin de sa mère. L'analyse détaillée des aberrations chromosomiques donnait quelques résultats atypiques qui sont examinés. La mère était soumise elle aussi à des examens cytogénétiques.

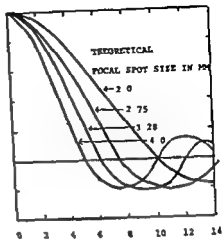
REFERENCES

- BENDER M. A. and GOOCH P. C. Persistent chromosome aberrations in irradiated human subjects. II. Three and one half year investigation. *Radiat. Res.* 18 (1963) 389.
 BUCKTON K. F., COURT BROWN W. M. and SMITH P. G. Lymphocyte survival in men treated with X rays for ankylosing spondylitis. *Nature* 214 (1967) 170.
 — JACOBS P. A., COURT BROWN W. M. and DOLL R. A study of the chromosome damage persisting after X ray therapy for ankylosing spondylitis. *Lancet* 1962 II p. 676.

MTF



a



b

Fig 1 a) MTF curves of a typical roentgen ray focal spot measured experimentally along the central axis in four different directions on a plane normal to the direction of the incident beam parallel to the projected width of the focal spot (marked as 0) 45 to it 90 to it and 135 to it The focus-object distance was 100 cm and the object-film distance was 5 cm. b) Theoretical MTF curves of a square focal spot of different sizes at a focus-object distance of 100 cm and object-film distance of 5 cm

various reasons it is important to be able to measure the MTF of roentgen ray focal spots experimentally. Methods of making these measurements have been described by KANAMORI (1965), DOI et al (1965) and by GOPALA RAO & BATES (1968). Using these methods it is possible to generate for each focal spot a family of MTF curves at various positions, distances and angulations.

Although the above mentioned method of describing the resolution characteristics of a roentgen ray focal spot is extremely rigorous from a physicist's point of view, a practising radiologist interested in the intercomparison of several different roentgen tubes will find the use of families of MTF curves rather impractical. From his point of view, a direct comparison of the linear dimensions of the focal spots is much simpler. Yet, as demonstrated by GOPALA RAO & BATES (1968), resolution cannot be judged adequately from the nominal focal spot size as quoted by the manufacturers. This difficulty can be overcome if one calculates the effective dimensions of a focal spot from a knowledge of the experimentally measured modulation transfer functions.

In Fig 1a the modulation transfer functions of a typical roentgen ray focal

EFFECTIVE DIMENSIONS OF ROENTGEN TUBE FOCAL SPOTS BASED ON MEASUREMENT OF THE MODULATION TRANSFER FUNCTION

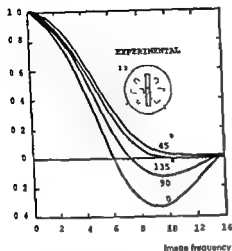
by

GOPALA U V RAO and LLOYD M BATES

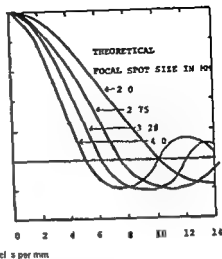
In recent years, the modulation transfer function (MTF) has become a widely accepted concept for the specification of the resolution capabilities of roentgen imaging systems. One of the important causes of loss of resolution in these systems is the finite size and shape of the focal spot. Several years ago, MORGAN (1962) derived a theoretical expression for the modulation transfer function of a square focal spot with uniform emission.

The MTF of a focal spot can be calculated from theory alone, only if it can be assumed that the focal spot is of a well defined geometric shape, such as a square, a rectangle or two parallel bars. However roentgen ray focal spots in general are not composed of such perfect geometric shapes nor do they have uniform emission. Furthermore, since the roentgen ray focal spots are generally designed on the line focus principle, the size of the projected focal spot varies with the angle of projection. Hence radiographic resolution or the MTF also depends on the position and direction of interest in a plane normal to the central axis of the beam. This effect is further accentuated by the fact that the shape of the focal spot also varies with the angle of projection. For these

MTF



a



b

Fig 1 a) MTF curves of a typical roentgen ray focal spot measured experimentally along the central axis in four different directions on a plane normal to the direction of the incident beam parallel to the projected width of the focal spot (marked as 0) 45 to 90 to 135 to 180. The focus-object distance was 100 cm and the object-film distance was 5 cm. b) Theoretical MTF curves of a square focal spot of different sizes at a focus-object distance of 100 cm and object-film distance of 5 cm.

For various reasons it is important to be able to measure the MTF of roentgen ray focal spots experimentally. Methods of making these measurements have been described by KANAMORI (1965) DOI et al (1965) and by GOPALA RAO & BATES (1968). Using these methods it is possible to generate for each focal spot a family of MTF curves at various positions, distances and angulations.

Although the above mentioned method of describing the resolution characteristics of a roentgen ray focal spot is extremely rigorous from a physicist's point of view, a practising radiologist interested in the intercomparison of several different roentgen tubes will find the use of families of MTF curves rather impractical. From his point of view, a direct comparison of the linear dimensions of the focal spots is much simpler. Yet, as demonstrated by GOPALA RAO & BATES (1968), resolution cannot be judged adequately from the nominal focal spot size as quoted by the manufacturers. This difficulty can be overcome if one calculates the effective dimensions of a focal spot from a knowledge of the experimentally measured modulation transfer functions.

In Fig 1a the modulation transfer functions of a typical roentgen ray focal

Table 1
Effective dimensions of a typical focal spot

λ	0		45		90°		135	
	Freq at which the MTF is (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is (cycles/mm)	Eff focal spot size in mm
0.8	2.2	3.27	3.0	2.40	2.6	2.77	2.7	2.67
0.6	3.4	3.12	4.4	2.41	4.0	2.65	4.1	2.59
0.4	4.3	3.16	5.6	2.43	5.0	2.72	5.3	2.57
0.2	5.2	3.19	7.1	2.34	6.0	2.77	6.7	2.48
0.0	6.1	3.28	—	—	7.3	2.74	—	—
	Mean focal spot size 3.20		2.40		2.73		2.58	

spot, measured along the central axis in four different directions on a plane normal to the direction of the incident beam are recorded parallel to the projected width of the focal spot (marked as 0° in the figure), 45° to it, 90° to it and 135° to it. In Fig. 1 b are shown the theoretical MTF curves for various sizes of focal spots, calculated using the standard theoretical formula (MORAN 1962) for the MTF of a square roentgen ray focal spot

The modulation transfer function $M(f_i)$ of a square focal spot of projected width a is given by the expression

$$M(f_i) = \frac{\sin(\pi f_i a / d_i)}{(\pi f_i a / d_i)}$$

where f_i is the spatial frequency in cycles per millimeter in the image plane of a test object with sinusoidal transmission placed at a distance of d_i from the focal spot and a distance d_o from the film

Looking at the curve for zero degrees in Fig. 1 a, one observes that the MTF passes through zero at an image frequency of 6.1 cycles/mm. Looking at the theoretical curves on the right hand side, it is seen that the MTF of a square focal spot of 3.28 mm also passes through zero at the same spatial frequency. It follows therefore, that as far as the zero degree position is concerned, the roentgen tube in question may be said to have an effective focal spot size of 3.28 mm.

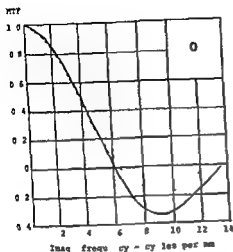


Fig 2 Diagram showing the agreement between the experimental MTF curve (solid) and that calculated theoretically using the concept of a mean effective size (dotted). The curves shown refer to the 0 position of fig 1

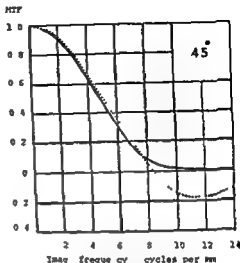


Fig 3 Diagram showing the agreement between the experimental MTF curve (solid) and that calculated theoretically using the concept of mean effective size (dotted). The curves shown refer to the 45° position in fig 1

The MTF of a theoretical square focal spot of size a passes through zero at an image frequency f given by the equation $f_1 = d_1/ad$ (see formula p 364). Therefore in general if the MTF obtained experimentally at a focus to object distance d_1 and an object to film distance d passes through zero at an image frequency $(f)_0$ an effective focal spot size can be calculated using the equation

$$\text{eff size} = \frac{1}{(f)_0} \frac{d_1}{d_2} \quad (1)$$

In a similar manner it is possible to compare the frequencies at which the experimental and theoretical MTF curves have similar values other than zero for example say 0.2. According to the theoretical equation for a square focal spot (see formula p 364) the MTF becomes equal to 0.2 when $f_1 = 0.83 d_1/ad$. This means that if an experimentally measured MTF curve has a value of 0.2 at an image frequency $(f)_0$ an effective focal spot size can be calculated using the equation

$$\text{eff size} = \frac{0.83}{(f)_{0.2}} \frac{d_1}{d_2} \quad (2)$$

Table 1
Effective dimensions of a typical focal spot

λ	0		45		90		135	
	Freq at which the MTF is \ (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is \ (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is \ (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is \ (cycles/mm)	Eff focal spot size in mm
0.8	2.2	3.27	3.0	2.40	2.6	2.77	2.7	2.67
0.6	3.4	3.12	4.4	2.41	4.0	2.65	4.1	2.59
0.4	4.3	3.16	5.6	2.43	5.0	2.72	5.3	2.57
0.2	5.2	3.19	7.1	2.34	6.0	2.77	6.7	2.48
0.0	6.1	3.28	—	—	7.3	2.74	—	—
	Mean focal spot size	3.20		2.40		2.73		2.58

spot, measured along the central axis in four different directions on a plane normal to the direction of the incident beam are recorded parallel to the projected width of the focal spot (marked as 0° in the figure), 45° to it, 90° to it and 135° to it. In Fig. 1 b are shown the theoretical MTF curves for various sizes of focal spots, calculated using the standard theoretical formula (MORGAN 1962) for the MTF of a square roentgen ray focal spot.

The modulation transfer function $M(f_i)$ of a square focal spot of projected width a is given by the expression

$$M(f_i) = \frac{\sin(\pi f_i a d_2 / d_1)}{(\pi f_i a d_2 / d_1)}$$

where f_i is the spatial frequency in cycles per millimeter in the image plane of a test object with sinusoidal transmission placed at a distance d_1 from the focal spot and a distance d_2 from the film.

Looking at the curve for zero degrees in Fig. 1 a, one observes that the MTF passes through zero at an image frequency of 6.1 cycles/mm. Looking at the theoretical curves on the right hand side, it is seen that the MTF of a square focal spot of 3.28 mm also passes through zero at the same spatial frequency. It follows therefore, that as far as the zero degree position is concerned, the roentgen tube in question may be said to have an effective focal spot size of 3.28 mm.

The effective dimensions of the focal spot of Fig 1 are given in Table 1, in four directions on a plane normal to the central axis of the beam. These were calculated using equation (3) above for various values of x ranging from 0 to 0.8. The last column of the table shows the mean effective focal spot dimensions in each of these four directions. The experimental MTF curve and that calculated theoretically for the 0° position using the mean effective size, are recorded in Fig 2. The agreement between the theoretical and the experimental curves in the case of the 45° position is apparent from Fig 3.

The mean effective focal spot dimensions of several different roentgen tubes are presented in Table 2. In general, the effective focal spot dimensions as calculated from experimentally measured MTF data may be seen to be considerably larger than the nominal size claimed by the manufacturers. They also depend to a considerable extent on the direction of interest in a plane normal to the central axis of the incident beam.

Conclusion

Above has been presented a method of calculating the mean effective dimensions of roentgen ray focal spots on the basis of experimentally measured modulation transfer function data. The method described is not rigorous in the sense that it assumes that experimentally measured modulation transfer functions of any shape can be matched with the theoretical curves for a linear spot of appropriate dimensions. It may be argued that this assumption is not always quite valid. However, we have found that the method yields extremely satisfactory agreement between the experimental and the theoretical curves except in the region of spatial frequencies when the MTF becomes negative (see Fig 2). This however is of no serious practical significance, since the region of negative MTF is important only when strictly periodic patterns are imaged. As far as the imaging of finite structures is concerned the region of negative MTF does not contribute significantly to image deterioration because the absolute values of the MTF are usually very small in this region.

SUMMARY

A method is described for the calculation of mean effective dimensions of a roentgen ray focal spot based on an experimental measurement of the modulation transfer function.

ZUSAMMENFASSUNG

Eine Methode wird beschrieben die mittlere effektive Grösse des Fokus Flecken von Röntgenstrahlen auf der Basis von experimentellen Messungen der Modulation der Transfer Funktion abzuschätzen.

Table 2

Mean effective dimensions of the focal spots of four typical roentgen ray tubes expressed in millimeters

Tube No	Small focal spots					Large focal spots				
	Nominal size according to manufacturer	Mean effective size				Nominal size according to manufacturer	Mean effective size			
		0	45	90	135		0	45	90	135
1	0.5	1.44	1.15	0.82	1.15	1.5	2.14	1.63	1.98	1.71
2	1.0	1.65	1.41	1.54	1.41	2.0	2.69	2.57	2.72	2.61
3	1.0	1.79	1.32	1.55	1.35	2.0	3.20	2.40	2.73	2.58
4	1.0	1.68	1.40	1.51	1.41	2.0	2.95	2.41	2.73	2.59

In general, if an experimentally obtained MTF curve has a value x at frequency $(f_i)_x$, an effective focal spot size can be calculated using the equation

$$\text{eff size} = \frac{\tau_x}{(f_i)_x} \frac{d_1}{d_2} \quad (3)$$

x	τ_x
0	1.00
0.2	0.83
0.4	0.68
0.6	0.53
0.8	0.36

where τ_x is a constant that depends on x

The value of τ_x for any particular value of x is simply the normalized frequency f/f_1 (f_1 being equal to d_1/ad_2) at which the MTF of a focal spot calculated from MORAN's theoretical expression becomes equal to x . In other words x and τ_x are related to each other by the equation

$$x = \frac{\sin(\pi\tau_x)}{(\pi\tau_x)}$$

If x is selected as zero, $\tau_x = 1.0$ and equation (3) obviously reduces to equation (1). Likewise if x is selected as 0.2, τ_x equals 0.83 and equation (3) reduces to equation (2).

The use of the generalized equation can be illustrated by a further example. Looking at Fig. 1 again, we find that the MTF in the case of the 135° position has a value of 0.2 at an image frequency of 6.7 cycles/mm. Substituting $(f_i)_x = 6.7$ and $\tau_x = 0.83$ in equation (3), the effective focal spot size in this case can be seen to be $\frac{0.83}{6.7} \times \frac{100}{5}$ or 2.48 mm.

MODIFYING EFFECTS OF CYSTEAMINE ON EXPERIMENTAL RADIATION LESIONS OF THE BRAIN

by

OVE HÄSSLER

Compounds that afford protection against the effects of ionizing radiation have become of great biological and military importance in recent years. It has, for instance, been estimated that if the compound at present most commonly used for protection (cysteamine) could be administered effectively in connection with a total nuclear attack on the USA 12 million lives would be saved (NELSON & ÅKERFELDT 1967) and even more effective protective compounds are under investigation.

Compounds administered to protect against radiation are absorbed in varying degrees by the various tissues (NELSON & ULLBERG 1960). It can therefore be expected that radioprotective agents affect the different tissue components to different extents so that radiation damage can be modified selectively by choosing between the various protective agents. The objective of the present work has been to study whether and how a radiation lesion of the brain may be modified by cysteamine. Knowledge of such modifications may be of use in radiation treatment of malignant tumours and other disorders and should also increase the understanding of the pathomechanisms of a radiation lesion.

This work was supported by Grant No. B69 12X 561 05 from the Swedish Medical Research Council. Submitted for publication 23 September 1969.

RÉSUMÉ

Les auteurs décrivent une méthode pour calculer les dimensions moyennes efficaces de la tache focale d'un tube à rayon de roentgen méthode basée sur la mesure expérimentale de la fonction de transfert de modulation

REFERENCES

- DOI K, KAJI TAKIZAWA T and SAYANAGI K Application of optical transfer function in radiography Jap J appl Phys (1965) Suppl No 1, p 183
- GOPALA RAO U V and BATES L M The modulation transfer functions of X ray focal spots Phys in Med Biol 14 (1969) 93
- KANAMORI H Optical transfer function of X ray tube focal spots for various tube currents Jap J appl Phys 4 (1965) 227
- METHODS OF EVALUATING RADIOLOGICAL EQUIPMENT AND MATERIALS Recommendations of ICRU Report 10f (1962) NSB Handbook No 89 (1963)
- MORGAN R H The frequency response function A valuable means of expressing the information recording capability of diagnostic X ray systems Amer J Roentgenol 88 (1962) 175

Table

Distribution of various types of radiation lesions in fields irradiated with and without cysteamine. In group I the dose administered was the same to both fields (2 300 rad 1 cm below the skin in the brain) while in group II the dose to the cysteamine field was 50% higher (3 450 rad). A = animals with no lesions B = animals with lesion in the control field only C = animals with lesion in the cysteamine field only D = animals with lesions in both fields

	Group I (ten rabbits)				Group II (ten rabbits)			
	A	B	C	D	A	B	C	D
Inflammation of skin	2	4	0	4	1	0	2	7
Skin erosions	5	3	0	2	2	0	3	5
Complete epilation	7	3	0	0	6	0	2	2
Incomplete or complete epilation	1	7	0	2	0	0	0	10
Incomplete hair covering when killed	1	2	0	7	0	0	1	9
Hypertrophies	3	5	0	2	1	0	3	6
Skin atrophy	1	5	0	4	1	0	0	9
Telangiectases in white matter	4	4	0	2	2	0	2	6
Vascular telangiectases in white matter	8	2	0	0	7	0	2	1
Telangiectases in grey substance	8	2	0	0	7	0	2	1
Meningeal fibrosis	10	0	0	0	7	0	3	0
Meningeal inflammation	8	2	0	0	4	1	3	2
Fibrosis in intracranial walls	9	1	0	0	5	0	3	2
Fibrosis of choroid plexus	10	0	0	0	6	0	3	1
Inflammation of the choroid plexus	10	0	0	0	8	0	2	0
Haemorrhage	5	4	1	0	4	1	2	3
Large haemorrhage	10	0	0	0	9	0	1	0
Endothelial proliferation and swelling	6	3	1	0	5	0	2	3
Fibrosis in cross of arterial wall	8	2	0	0	6	0	3	1
Hyalinosis of arterial wall	6	4	0	0	5	0	2	3
Periarterial inflammation	0	3	1	0	5	1	2	2
Plasmatic impregnation	8	2	0	0	8	0	2	0
Fat granule cell	0	4	0	0	5	0	3	2
Fibrous capsule of the cerebral parenchyma	9	1	0	0	0	0	2	2
Calcifications	6	4	0	0	5	0	3	2
Amyloid like material	9	1	0	0	6	0	2	1
Necrotic cysts in white matter	6	4	0	0	5	0	3	2
Large necrotic cysts or cysts in white matter	8	2	0	0	7	0	1	2
Necrotic cysts in grey substance	8	2	0	0	7	0	1	2
Loss of normal architecture	3	5	0	2	1	0	4	5
Destruction of myelin sheaths	0	5	1	4	0	0	3	7
Gliosis	6	4	0	0	3	0	4	3
Atrophic hypotrophy	10	0	0	0	7	1	1	1
Atypical microglia	9	1	0	0	8	0	2	0
Fibrillar gliosis	9	1	0	0	8	1	1	0
Elongated dendroglia	10	0	0	0	8	0	2	0
PAS positive granule cell dendroglia	8	2	0	0	4	0	4	2
Spontaneous neuropil myelins	10	0	0	0	9	0	0	1

Materials and Methods

Autoradiographic study in mice with ^3H thymidine Twelve adult male white mice of an inbred strain (N M R I, Bethesda, supplied by Anticimex, Norrviken, Sweden), belonging to two litters and weighing 18 to 22 g at the start of the experiment, were employed. One (control) cerebral hemisphere was irradiated by roentgen rays as in a previous work (HÄSSLER 1966). Within 5 minutes after this irradiation 5 mg of cysteamine HCl (Th. Schuchardt GmbH, Munich, West Germany) were dissolved in sterile water, neutralized with dilute NaOH to pH 7, and injected intraperitoneally into each mouse a few minutes before a second irradiation which was performed in the same way as the first one but symmetrically on the opposite hemisphere. Thus, one (control) hemisphere was irradiated without and the other with administration of cysteamine. The doses were the same in both hemispheres (2370 ± 237 rad 4 mm below the skin). All the animals were killed 7 days after irradiation.

The autoradiographic study with ^3H thymidine (supplied by the Radiochemical Centre, Amersham, England), the histologic investigation, and the counting of labelled cells, were performed in the same way as in a previous work (HÄSSLER 1966).

Microangiographic and histologic studies in rabbits Twenty male rabbits (Swedish Landrace) weighing 1.5 to 2.0 kg at the time of irradiation, were used. They were divided into two groups according to the doses of radiation administered. In all the animals, one (control) cerebral hemisphere was irradiated by ^{60}Co as in a previous study (HÄSSLER & MOVIN 1966). Within 5 minutes following irradiation, a solution of cysteamine (200 mg/kg body weight), prepared freshly as above, was injected intravenously, and the opposite hemisphere was irradiated a few minutes later in a similar way.

In the first ten animals, group I, the dose to the opposite hemisphere was the same as in the control hemisphere (2300 ± 230 rad in the brain 1 cm below the skin). In the remaining ten animals, group II, the dose to the opposite hemisphere was 50% higher (3450 ± 345 rad 1 cm below the skin), in order to obtain a similar effect in both hemispheres.

The animals were examined for skin reactions (see Table) every third day for 10 to 40 days following irradiation, thereafter the examinations were performed only once a week. When complete epilation occurred within an area of at least 0.75 cm², the irradiation field was tribulated under complete epilation.

All the animals, except two, were killed 360 days after irradiation. The two animals (both belonging to group II) became ill and lost appetite and weight, when their condition was judged to be hopeless, they were killed (274 and 309 days after irradiation respectively). Microangiography of all the twenty animals

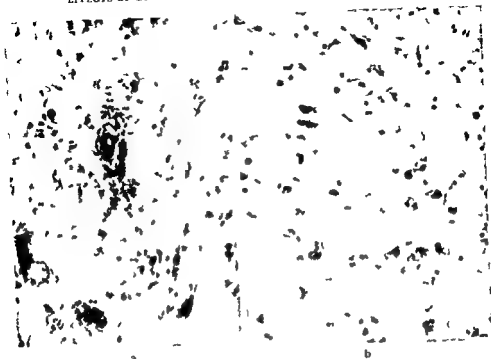


Fig. 2. Histologic sections through small representative parts of the sections shown in fig. 1a and 1b respectively. $\times 178$. The histologic changes correspond well with the angiographic appearances.

All the slides were assessed as unknown (a blind study) when the histologic changes (cf. Table) were graded. If the soft meninges were so fibrosed and thickened that they were more than $100\ \mu$ in the sections, this was recorded in the table. When more than eight inflammatory cells occurred in the meninges of one section this was registered as meningeal inflammation. Fibrosis of the plexus choroideus was also recorded when it occupied an area exceeding $0.5\ \text{mm}^2$ in one section. Inflammation of the choroid plexus was registered when more than eight inflammatory cells occurred in one section. Large haemorrhage indicates bleeding exceeding $1\ \text{mm}^2$. Hyalinosis of the arterial wall was diagnosed only when all muscle cells had disappeared in an artery greater than $100\ \mu$ in diameter. Perivascular inflammation was registered when at least ten inflammatory cells were seen around the vessels in a section. Fibrous scars in the cerebral parenchyma had to occupy at least $0.2\ \text{mm}^2$ in one section to be recorded. Large necrosis or cyst was diagnosed when the total volume exceeded $1\ \text{mm}^3$. The normal architecture had to be lost within $4\ \text{mm}^2$ in one section.

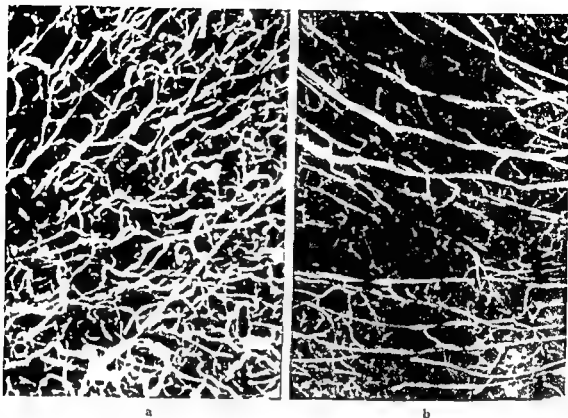


Fig 1 Microangiograms from cross sections through the dorsal superior part of the brain stem of rabbit group I a) Left side (control) irradiated with ^{60}Co γ rays 2 300 rad $\times 43$ b) Right side Cysteamine was injected intravenously immediately before the same dose of ^{60}Co γ rays was given $\times 43$ The hemisphere protected by cysteamine had fewer angiographic changes

was performed as in a preceding study (HASSLER & MOVIN 1966) Teleangiectases also were measured as in that study, if longer than 1 mm, they were classified as marked teleangiectases

Five slices in the frontal plane from each cerebral hemisphere were embedded in paraffin, these were cut from the same parts of the brain in all the animals and so that as many parts of the brain as possible were represented Eight sections from each slice were prepared and stained with van Gieson's stain, haematoxylin-eosin, Nissl's stain, PAS and McMahon's myelin stain as well as by Ranke's Victoria blue for astrocytes (SILVERTON & ANDERSON 1961), Palmgren's silver technique for axons (PALMGREN 1948), and Ladevig's modification of the Mallory stain (ROULET 1948) When precipitations were seen, sections stained with von Kossa's stain and Turnbull blue (ROMEIS 1948) were also prepared and when amyloid like material was encountered, the alkaline Congo red procedure (PUCHTLER, SWFAT & LEVINE 1962) was applied

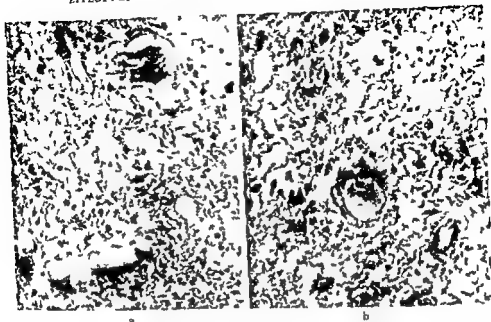


Fig 4 Histologic sections through representative parts of the sections shown in fig 3a and 3b respectively $\times 118$. The angiographic and histologic changes were equally marked on both sides.

of variations in thickness of the sections. When the number of labelled cells of various types on the side irradiated with cysteamine in each section was calculated as a percentage of the corresponding number from the control irradiated side these sources of error were eliminated. It could then be observed that a significant difference ($p < 0.01$) existed in the largest group (small and dark cells).

Microangiographic and histologic studies in the rabbit. All the animals, with the exception of the two killed earlier than one year of observation, gained in weight from 2.9 to 4.3 kg. Several animals, especially in group II, had various neurologic signs (anisocoria, vestibulo-cerebellar disturbances, wry neck). Abscesses, tumours or marked hydrocephalus were never noted.

The distribution of the skin reactions given in the Table reveals marked inter-individual variations, although much smaller variations occurred in the individuals. The cysteamine side in group I was always less changed than the control side, while the opposite occurred in group II. The hair that grew after epilation in the pigmented rabbits was usually whitish grey.

The results of microangiography (telangiectases in the Table) were in good

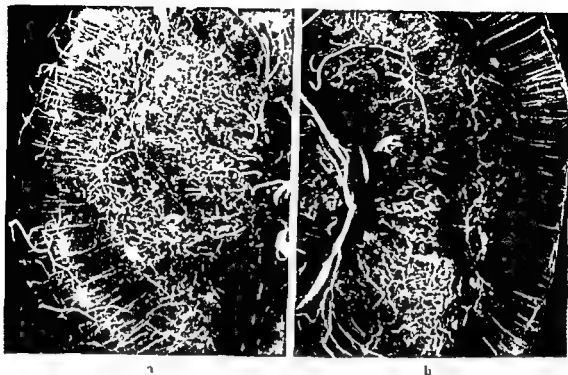


Fig 3 Microangiograms from 2 mm thick coronal slices through the posterior part of the brain of rabbit group II $\times 8$ a) Left side (control) irradiated with ^{60}Co γ rays (300 rad) b) Right side which after injection of cysteamine received 450 rad ^{60}Co radiation Both sides had marked vascular changes with teleangiectases mainly in the white matter

in order to be included. Glial foci had to comprise at least five cells in close contact with each other to be counted.

Results

Autoradiographic study in mice No animal died spontaneously or presented any changes during the week between irradiation and killing. No marked abnormalities could be discovered in the histologic sections except for some pyknotic cells in the radiosensitive subependymal layer bilaterally. Small and dark cells, interpreted as precursors to oligodendroglia cells, occurred in the hemisphere irradiated without cysteamine, numbering a total of 18.6 ± 3.1 labeled cells in the counted area of each section while in the hemisphere irradiated with cysteamine they numbered 13.7 ± 2.8 . The corresponding figures for the subependymal cells were 3.8 ± 1.0 and 5.1 ± 1.4 respectively. The figures for the microglia cells, the endothelial cells and the unclassified cells, which were all grouped together, were 3.5 ± 1.6 and 5.0 ± 1.8 , respectively. The figures for the standard deviation of the values were comparatively high because

in whole body autoradiography is low). The second explanation would seem to be supported by the results of the present autoradiographic studies which indicated that the cells incorporating ^3H thymidine are protected by the cysteamine so that more normal values are obtained (cf. HASSLER 1966) when cysteamine is administered at the same time as the irradiation.

The present investigation indicates that after the administration of cysteamine the dose of radiation cannot be increased by as much as 50 % if the same effect on the brain and skin as without cysteamine is desired. This is not quite in accordance with observations regarding changes in mean lethal doses after the administration of cysteamine because the dose could then be further raised by 86 % (BACQ 1954) or by 67 % (NELSON 1965). The difference could in part be due to the unavoidable measuring inexactitude in all such work and to differences in the doses and animal species. Another explanation could be that the mechanisms determining the lethality in total body irradiation are more affected by cysteamine than are the skin vessels and the brain.

When the present work was planned it was believed that irradiation with cysteamine might produce a lesion different from those ordinarily associated with radiation. This was not proved, however. The unavoidable individual variations are great in all irradiation work and cause as large variations as cysteamine. Within the individuals the variations are much smaller. It therefore would seem to be advantageous to have the field irradiated with cysteamine and the control field without in one and the same animal. Inter individual variations can then be avoided.

SUMMARY

The modifying effect of cysteamine on experimental radiation lesions in the brain was studied in 12 mice and 20 rabbits. One cerebral hemisphere was irradiated before and the other immediately after the administration of cysteamine. The radioprotective effect of cysteamine was almost equally great in skin vessels and the brain parenchyma but less than that measured in the mean lethal dose in total body irradiation.

ZUSAMMENFASSUNG

Der modifizierte Effekt von Cysteamine auf experimentelle Strahlungsschaden im Gehirn wurde bei 12 Mäusen und 20 Kaninchen untersucht. Eine Gehirnhälfte wurde vor und die andere unmittelbar nach der Injektion von Cysteamine bestrahlt. Der strahlungsschützende Effekt von Cysteamine war ungefähr gleich gross in der Haut, den Gefässwänden und dem Gehirngewebe, aber geringer als der in durchschnittlich tödlicher Dosis gemessene nach Totalkörperbestrahlung.

agreement with the observations on the skin and with the histologic findings. The inter individual variations were much greater than those in the individuals themselves also as regards the telangiectases. Cystic defects in the brain parenchyma were generally closely associated in the angiograms with the telangiectases. Both the telangiectases and the cystic defects occurred mainly in the white matter, and when exceptionally large, also interfered with the grey substance. The same rabbits had marked telangiectases in the white matter and telangiectases in the grey substance. The size of the cystic defects correlated well with the size of the telangiectases.

As may be seen from the Table, the vascular changes seen in the histologic sections had a similar distribution as the changes observed in the skin and in the microangiograms. This also applies to the changes in the cerebral parenchyma. All were more marked in the white than in the grey matter. No qualitative differences could be discovered in the tissues irradiated with cysteamine. Signs of so called spontaneous encephalomeningitis were noted in only one rabbit. The changes were weak to moderate and because they were practically equally developed on both sides, they were presumed not to have influenced the results. The rabbit belonged to group II, had bilateral meningeal inflammation, no inflammation in the choroid plexus and no endothelial proliferation or swelling.

Discussion

It was assumed for several reasons when the present work was started that radiation lesions in the vessels but not in the cerebral parenchyma could be diminished by the administration of cysteamine. It was recognized that cysteamine has almost no effect on the acute radiation sickness, with cerebral symptoms and coma, following excessively high doses of total body irradiation (RUGH & CLUGSTON 1954, MAISIN & DOHERTY 1960, SORBO 1965). It was also known that ³S cysteamine is incorporated to a moderate extent into the mouse skin and blood vessels and to a very small degree into the brain (NELSON & ULLBERG 1960).

The administration of cysteamine in the present work appeared to diminish the lesions equally in the blood vessels and the brain parenchyma. Two different explanations may be offered for this. The changes in the brain parenchyma may in some measure be secondary to those in the vessels, as has been suggested by several investigators (cf. BERG & LINDGREN 1958). Cysteamine would work mainly in the vessels, inhibiting local changes and secondarily inhibiting those in the cerebral parenchyma. The other possible explanation is that cysteamine is incorporated into strategically important parts of the brain, for example the mitotically active glia cells (although the total incorporation in the brain visible

~~International Commission~~ on Radiation Units and Measurements

REPORT TO THE INTERNATIONAL EXECUTIVE COMMITTEE OF THE NINE INTERNATIONAL CONGRESS OF RADIOLOGY TOKYO 1969

For the ~~duration~~ of the actual program with which the ICRU has been engaged since the NINE International Congress of Radiology it would seem appropriate to submit a report of the results of the work which was reported to the International Executive Committee in 1962 and the ICRU efforts between 1962 and 1969 resulted in the publication of six major reports.

- ICRU Report 15a — Radiation quantities and units (published as NBS Handbook 44)
- ICRU Report 15b — Physical aspects of irradiation (published as NBS Handbook 62)
- ICRU Report 15c — Radioactivity (published as NBS Handbook 80)
- ICRU Report 15d — Clinical dosimetry (published as NBS Handbook 87)
- ICRU Report 15e — Radiobiological dosimetry (published as NBS Handbook 86)
- ICRU Report 15f — Methods of evaluating radiological equipment and materials (published as NBS Handbook 89)

Judging from the sales of these reports the work represented by them has been widely accepted in the scientific community. Even today the demand for these reports continues. Further the other publications resulting from Commission activities (See Annex) have also received wide dissemination.

Substructure and mode of operation adopted in 1962

In the report submitted to the International Executive Committee in 1962 it was pointed out that the period from 1962 to 1965 was devoted principally to elaboration of the substructure and mode of operation adopted by the Commission in 1962 and to the utilization of that substructure for the initiation of new projects. The period since the 1962 report has been utilized to carry forward the projects initiated with the new substructure. In addition the Commission made one significant modification in the mode of operation during this time period. This was the establishment in 1968 of the ICRU publications program with which the Commission began the publication of ICRU reports under its own auspices.

Activities during the period 1965 to 1969

The time period from 1965 through 1969 is particularly significant because a number of ICRU reports were completed during this period. In 1968 the Commission completed and published as the first reports available from the ICRU Publications Office

RÉSUMÉ

L'auteur a étudié sur 12 souris et 20 lapins l'effet modificateur de cystéamine sur les radio lésions expérimentales du cerveau. Un hémisphère cérébral a été irradié avant et l'autre immédiatement après l'administration de cystéamine. L'effet radio protecteur de la cystéamine a été presque aussi important au niveau de la paroi des vaisseaux et du parenchyme cérébral mais a été moindre que l'effet mesuré sur la dose létale moyenne au cours de l'irradiation totale du corps.

REFERENCES

- BACQ J. M. The amines and particularly cystamines as protectors against roentgen rays. *Acta radiol* 41 (1954) 47.
- BERG N. O. and LINDGREN M. Time dose relationship and morphology of delayed radiation lesions in the brain in rabbits. *Acta radiol* (1958) Suppl. No. 167.
- HASSLER O. Incorporation of tritiated thymidine into mouse brain after a single dose of X rays. An autoradiographic study. *J Neuropath exp Neurol* 25 (1966) 97.
- and MOVIN A. Microangiographic studies on changes in the cerebral vessels after irradiation. I. Lesions in the rabbit produced by ^{60}Co gamma rays 195 kV and 34 MV roentgen rays. *Acta radiol Ther Phys Biol* 4 (1966) 279.
- MAISEN J. R. and DOHERTY D. G. Chemical protection of mammalian tissues. *Federation Proc* 19 (1960) 564.
- NELSON A. Verkan av strålskyddsmidlen mot somatiska och genetiska strålskador. (In Swedish) *Nord Med* 74 (1965) 1183.
- and ULLBERG B. Distribution of S^{35} in mice after injection of S^{35} cysteamine. An autoradiographic investigation. *Acta radiol* 53 (1960) 305.
- NELSON A. and ÅKERFELDT S. Kemiskt strålskydd kan öka chansen att överleva atomkrig. (In Swedish) *Foatidningen* 2 (1967) 3.
- PALMGREN A. A rapid method for selective silver staining of nerve fibres and nerve endings in mounted paraffin sections. *Acta zool* 29 (1948) 377.
- PUCHTLER H. SWEAT F. and LEVINE M. On the binding of Congo red by amyloid. *J Histochem Cytochem* 10 (1962) 355.
- ROWEIS B. *Mikroskopische Technik*. R. Oldenbourg Verlag München 1968.
- ROULET F. *Methoden der pathologischen Histologie*. Springer Wien 1948.
- ROUGH R. and CLUCSTON H. The time intensity relations of whole body acute X irradiation and protection by beta mercaptoethylamine. *Radiat Res* 1 (1954) 437.
- SILVERTON R. E. and ANDERSON M. J. *Handbook of Medical Laboratory Formulae*. Butterworths London 1961.
- SORBO B. Strålskyddsmidlen. Strålskyddsmidlenas kemi, farmakologi och verkningsmekanism. (In Swedish) *Nord Med* 74 (1965) 1182.

International Commission on Radiation Units and Measurements

REPORT TO THE INTERNATIONAL EXECUTIVE COMMITTEE OF THE Xth INTERNATIONAL CONGRESS OF RADIOLOGY TOKYO 1969

Before turning to the actual program with which the ICRU has been engaged since the Xth International Congress of Radiology it would seem appropriate to mention some of the results of the work which was reported to the International Executive Committee in 1962 and 1963. ICRU efforts between 1959 and 1967 resulted in the publication of six major reports:

- ICRU Report 10a — Radiation quantities and units (published as NBS Handbook 84)
- ICRU Report 10b — Physical aspects of irradiation (published as NBS Handbook 85)
- ICRU Report 10c — Radioactivity (published as NBS Handbook 86)
- ICRU Report 10d — Clinical dosimetry (published as NBS Handbook 87)
- ICRU Report 10e — Radiobiological dosimetry (published as NBS Handbook 88)
- ICRU Report 10f — Methods of evaluating radiological equipment and materials (published as NBS Handbook 89)

Judging from the sales of these reports the work represented by them has been widely accepted in the scientific community. Even today the demand for these reports continues. Further, the other publications resulting from Commission activities (See Annex) have also received wide dissemination.

Substructure and mode of operation adopted in 1962

In the report submitted to the International Executive Committee in 1965 it was pointed out that the period from 1967 to 1965 was devoted principally to elaboration of the substructure and mode of operation adopted by the Commission in 1962 and to the utilization of that substructure for the initiation of new projects. The period since the 1965 report has been utilized to carry forward the projects initiated with the new substructure. In addition the Commission made one significant modification in the mode of operation during this time period. This was the establishment in 1968 of the ICRU publications program with which the Commission began the publication of ICRU reports under its own auspices.

Activities during the period 1965 to 1969

The time period from 1963 through 1969 is particularly significant because a number of ICRU reports were completed during this period. In 1968 the Commission completed and published as the first reports available from the ICRU Publications Office

ICRU Report 11 — Radiation quantities and units

ICRU Report 12 — Certification of standardized radioactive sources

Completed in 1968 and now being prepared for publication are three additional reports formulated under the substructure adopted in 1962

ICRU Report 13 — Neutron fluence, neutron spectra and kerma

ICRU Report 14 — Radiation dosimetry γ rays and gamma rays with maximum photon energies between 0.6 and 50 MeV

ICRU Report 15 — Cameras for image intensifier fluorography

Nearing completion and to be considered by the Commission at its meeting in 1969 are the following reports

Specification of high activity gamma ray sources

Radiation dosimetry γ rays from 5 to 150 kV

Linear energy transfer

Measurement of absorbed dose at a point in a standard phantom

Methods of arriving at absorbed dose at any point in a patient

Radiation protection instrumentation and its application

In addition to the above reports the current reporting period has seen the completion of two additional reports prepared by the authors at the request of the ICRU

BROWNELL G L, BERMAN M and ROBERTSON J S Nomenclature for tracer kinetics
Int J appl Radiat 19 (1968) 249

MACINTYRE W J, FEDORUK S O, HARRIS C C et coll Sensitivity and resolution in radioisotope scanning *In Proceedings of the Symposium on Medical Radioisotope Scintigraphy International Atomic Energy Agency Vienna 1969*

Still in preparation in ICRU subgroups are reports on the following subjects

Measurements of low level radioactivity

Display characteristics for radioisotope scanning

Methods of assessment of dose in tracer investigations

Electron beam dosimetry

High energy and space radiation dosimetry

Methods of compensating for body shape and inhomogeneity and of beam modification for special purposes

Statement of the dose received

Modulation transfer function its definition and measurement

From these lists of reports it is obvious that the substructure and mode of operation adopted by the Commission in 1962 involved a very substantial effort by the Commission to meet the expanding need for ICRU recommendations. The substructure developed to meet these needs was adopted on an experimental basis to alleviate some of the problems associated with the expanded program required. Previously the Commission's attempt to administer and review the work of each of the working groups imposed a very considerable burden on the Commission itself. The need to concern itself with each detail which was inherent in such a scheme of operation when coupled with the procedure of completing all reports at one time subjected the Commission members to an intolerable work load if rigorous standards were to be maintained. As can be seen from the list of reports completed the substructure and mode of operation adopted in 1962 has now produced results. These indicate that the substructure and mode of operation while not perfect and in some cases rather cumbersome has to a substantial extent succeeded in alleviating many of the problems previously experienced.

L. H. Gray Medal

Another important action taken by the Commission during the period since the XIII Congress of Radiology was the implementation with the cooperation of the International Society of Radiology and the International Congress of Radiology of the Commission's decision to establish an ICRU medal honoring the late L. H. Gray former Vice Chairman of the ICRU and a recognized leader in radiation quantities and units and in radiobiology. The Commission determined that the medal will be awarded for outstanding contributions in the scientific fields of interest to the ICRU. It was decided that the medal may be awarded once in each four years although the scientific work on which the award of the medal is based need not have occurred during the four year period prior to the award. In 1967 and 1968 the Commission gave careful consideration to the many nominations received for the first award of the medal in 1969. The Commission then determined that the first recipient of the L. H. Gray medal is to be Dr Louis V. Spencer a physicist at the National Bureau of Standards and Professor of Physics at Ottawa University (Kansas). Dr Spencer will receive the award at the XIII International Congress of Radiology. The award to Dr Spencer is based on his long term study of the theory of charged particle penetration. Dr Spencer has been invited to give a scientific lecture to the ICRU and its guests during the International Congress of Radiology.

ICRU relationships with other organizations

One of the features of ICRU activity in the last few years has been the development of relationships with other organizations interested in the problems of radiation quantities, units and measurements. In addition to its close relationship with the ICRP and its financial relationships with the International Society of Radiology, the World Health Organization and the International Atomic Energy Agency, the ICRU has also developed relationships of varying intensity with several other organizations. Since 1955 the ICRU has had an official relationship with the World Health Organization (WHO) whereby the ICRU is looked to for primary guidance in matters of radiation units and measurement and in turn the WHO assists in the worldwide dissemination of the Commission's recommendations. In 1960 the ICRU entered into consultative status with the International Atomic Energy Agency. The Commission has a formal relationship with the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) whereby ICRU observers are invited to attend UNSCEAR meetings.

The ICRU and the International Organization for Standardization (ISO) informally exchange notifications of meetings and the ICRU is formally designated for liaison with two of the ISO technical committees. The ICRU also corresponds and exchanges final reports with the following organizations: Bureau International des Poids et Mesures, Council for International Organizations of Medical Sciences, Food and Agricultural Organization, International Council of Scientific Unions, International Electrotechnical Commission, International Labor Organization, International Union of Pure and Applied Physics, United Nations Educational, Scientific and Cultural Organization.

The Commission has found its relationship with all of these organizations fruitful and of substantial benefit to the ICRU program.

Relations with these other international bodies do not affect the basic affiliation of the ICRU with the International Society of Radiology.

Commission finances

Throughout most of its existence the ICRU has operated essentially on a voluntary basis with the travel and operating costs being borne by the parent organizations of the participants (only token assistance was originally available from the International Society of Radiology). Recognizing the impracticability of continuing this mode of operation on an indefinite basis operating funds were sought from various sources.

Prior to 1959 the principal financial assistance for the ICRU had been provided by the Rockefeller Foundation which supplied some \$11 000 to make possible various meetings. In 1960 the Rockefeller Foundation supplied an additional sum of some \$4 000 making possible a meeting of the Quantities and Units Committee in 1960. In 1959 the International Society of Radiology increased its contribution to the Commission providing \$3 000 for the period 1959 to 1962. For the period 1962 to 1965 this was again increased the Society providing \$5 000. In March 1969 the Commission received \$3 500 from the Society as the first increment of the 1965 to 1969 contribution. In July 1969 an additional \$4 000 was received.

In 1960 and 1961 the World Health Organization made available the sum of \$3 000 each year. This was increased to \$4 000 in 1962 and this amount has been made available annually since then. It is expected that this sum will be allocated annually at least for the next several years.

In connection with the Commission's Joint Studies with the ICRU the United Nations allocated the sum of \$10 000 for the joint use of the two Commissions.

The most substantial contribution to the work of the ICRU has come from the Ford Foundation. In December 1960 the Ford Foundation made available to the Commission the sum of \$37 000 per year for a period of five years. This grant was to provide for such items as the travel expenses involved in attendance at meetings, secretarial services and other operating expenses. In 1965 the Foundation agreed to a time extension of this grant making available for the period 1966 to 1970 the unused portion of the original grant. To a large extent it is because of this grant that the Commission has been able to move forward actively with its program.

In 1963 the International Atomic Energy Agency allocated the sum of \$6 000 per year for use by the ICRU. This was increased to \$9 000 in 1967. It is expected that this sum will be allocated annually at least for the next several years.

From 1934 through 1964 valuable indirect contributions were made by the US National Bureau of Standards where the Secretariat resided. The Bureau provided substantial secretarial services, publication services and travel costs in the amount of several thousands of dollars.

The Commission wishes to express its deep appreciation to all of these organizations and to the others that have contributed so importantly to its work.

Membership

In the period since the Xth International Congress of Radiology the election of two new Commission members was made necessary by the resignations of J. W. Borg and H. F. Johns. Elected to fill these vacancies were J. R. Greening and A. Tsuya.

Thus the current membership consists of the following:

A. Allisy France, R. H. Chamberlain USA, F. P. Cowman USA, J. F. Fowler United Kingdom, F. C. Grawert Germany, J. R. Greening United Kingdom, K. Lidén Sweden.

R H Morgan USA H H Rossi USA L S Taylor USA A Tsuya Japan M Tubiana France and H O Wyckoff USA

Serving as Commission Officers until the XIIth International Congress of Radiology are
L S Taylor Chairman M Tubiana Vice Chairman H O Wyckoff Secretary and
W R Ney Technical Secretary

Elected at the recent meeting of the Commission were

Member Emeritus and Honorary Chairman L S Taylor

New members 1969 to 1973 A M Kellerer Germany W K Sinclair USA F W Spiers United Kingdom and A Wambersie Belgium

Continuing members 1969 to 1973 A Allisy France F P Cowan USA F Gauwerky Germany J H Greening United Kingdom K Liden Sweden R H Morgan USA H H Rossi USA A Tsuya Japan and H O Wyckoff USA

Senior Advisors 1969 to 1973 R H Chamberlain F F Fowler and M Tubiana

Commission Officers H O Wyckoff Chairman A Allisy Vice Chairman K Liden Secretary and W R Ney Technical Secretary

Outlook for the future

On the basis of the ICRU's experience during the last four years the outlook for the future is bright indeed. The helpful response of all of the individuals and organizations interested in the Commission's program augurs well for the future. For the substantial help received from these individuals and organizations the Commission expresses its deep appreciation.

The activities of the currently active ICRU subgroups are progressing well and in the near future the Commission should be in a position to publish several new reports. Administratively the activities of the ICRU seem soundly based. Funding appears to be the most critical problem facing the Commission especially over the next two or three years. The availability of the Ford Foundation funds terminates by the end of 1970 but they will have been exhausted by about the middle of the year. In the meantime the Commission is embarking upon a plan to recoup some of its expenses through its publication program. This will entail raising the selling price of its reports but it is believed that this will not seriously cut down the wide dissemination of its recommendations. However it is expected that this source of income will meet the costs of operating the publications program for about two years. After that the publication is expected to provide appreciable funds for ICRU operations.

Thus the next two or three years will be financially critical. Strenuous efforts will have to be made by the Commission's present supporters and its friends to obtain some additional funding during that period. The only alternative will be to drastically curtail its efforts, even on programs that are approaching completion.

Nevertheless it is evident that in spite of the funding problems the current status of the Commission's program viewed in its entirety and in the long range justifies confidence in the future.

Respectfully submitted
Lauriston S Taylor

ANNEX

ICRU materials published outside of the ICRU Reports Series

- Exposure of man to ionizing radiation arising from medical procedures — An inquiry into methods of evaluation A report of the International Commission on Radiological Protection and the International Commission on Radiological Units and Measurements Phys in Med Biol 2 (1957) 107 (Italian translation Società Italiana di Radiologia Medica e di Medicina Nucleare Milan 1958)
- Method of focal spot image formation and measurement (For diagnostic tubes up to 150 kVp) Acta radiol 55 (1961) 75 Amer J Roentgenol 85 (1961) 191 National Bureau of Standards Handbook No 78 p 80 US Government Printing Office Washington 1961, Radiology 76 (1961), 121
- Exposure of man to ionizing radiation arising from medical procedures with special reference to radiation induced diseases — An inquiry into methods of evaluation A report of the International Commission on Radiological Protection and the International Commission on Radiological Units and Measurements Phys in Med Biol 6 (1961) 199
- Report of the RBE Committee to the International Commissions on Radiological Protection and Radiological Units and Measurements Hith Phys 9 (1963) 357
- BROWNELL G L, BERMAN M and ROBERTSON J S Nomenclature for tracer kinetics Int J appl Radiat 19 (1968) 249
- MACINTYRE W J, FEDORUK S O, HARRIS C C et coll Sensitivity and resolution in radioisotope scanning In Proceedings of the Symposium on Medical Radioisotopes Scintigraphy International Atomic Energy Agency Vienna 1969

HISTOPATHOLOGY OF LATE LOCAL RADIO- LESIONS IN THE GOAT BRAIN

by

BENGT ANDERSSON BORJE LARSSON LARS LEKSELL WILLIAM MAIR
BROR REXED PATRICK SOURANDER and JAN WENNERSTRAND

The use of stereotactically directed high energy proton beams for the production of small circumscribed lesions in the depth of the brain has been demonstrated in previous investigations by LARSSON et coll 1958 LEKSELL et coll 1960 MAIR et coll 1967 In view of the current use of such techniques for local destruction of brain tissue in man (LARSSON et coll 1963) it is desirable to study the late appearance of local radio-lesions in the brain The present paper describes qualitatively 6 lesions in the brain of goats 1 1/2 to 4 years after cross-fire irradiation with a beam of 185 MeV protons The techniques of irradiation and dosimetry were except for some variation of the physical parameters the same as previously described in a short term study (LEKSELL et coll 1960)

Material and Methods

Radiation technique Adult goats of unknown age were irradiated by cross fire irradiation with 20 to 22 fields The individual field size number of fields

From the Department of Radiobiology the Gustaf Werner Institute and the Department of Anatomy University of Uppsala Sweden This investigation was supported by grants from the Swedish Medical Research Council Submitted for publication 22 October 1969

Table
Physical parameters of irradiation

Lesion No	Time of irradiation (min)	Field size (mm ²)	Number of fields	Number of planes	Max angle (°)	Real centre dose (rad)	Killed at (day after irradiation)
1	115	7×2	21	2	20	20 000	573
2	59	7×2	21	2	20	20 000	757
3	61	7×2	21	2	20	20 000	781
4	119	7×2	21	2	20	20 000	845
5	45	10×3	21	2	20	15 000	1 170
6	47	7×2	21	2	20	20 000	1 453

angular coordinates, 'real centre dose' (cf I FAKSIL *et coll* 1960), and time of irradiation are given in the Table above.

Animal care The animals were kept for study under ordinary farming conditions. During May to October they stayed out of doors in an enclosed well drained pasture with free access to water and salt. During November to April they were kept in a shed on straw. In the latter period they were given hay, salt and water freely, and in addition about 200 g of oats daily per animal. The animals were regularly observed but no neurologic examinations were made. Animals that died or were killed due to intercurrent disease were excluded from the investigation.

Histologic methods Brains taken from apparently healthy animals were studied at different intervals. The animals were decapitated 1 1/2 to 4 years after irradiation under nembutal anesthesia, and the brain was perfused through the carotids with physiologic saline followed by 5 per cent formol saline. The fixed brains were cut sagittally in slices 10 mm thick and examined macroscopically. The target region was embedded in celloidin and cut serially at 15 μ . The following staining methods were used: Nissl, Woelke, Gomori, Romanow and Masson.

Results

Lesion 1 18 months 20 000 rad The lesion was situated in the optic chiasm and adjacent brain tissue (Fig 1 upper). It took the form of a large necrotic cavity with a crenated margin lined by macrophages. Several smaller foci of similar appearance were present in the tissue around the main necrotic

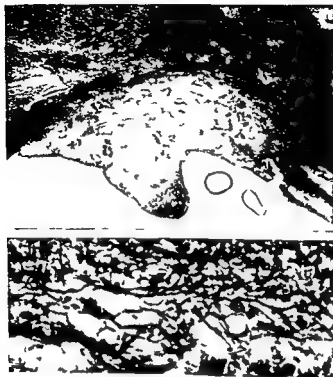


Fig 1 Lesion 1 18 months 20 000 rad Upper The lesion is sharply demarcated and lies in the optic chiasma and adjacent cerebral tissue Woelke $\times 8$ Lower Swollen axons in the optic chiasma Romanes $\times 400$

zone Increased vascularity with congested vessels perivascular round cell cuffing and proliferation of astrocytes were prominent around the cavity Some of the astrocytes were giant cells Some proliferation of astrocytes had also taken place in the damaged grey and white matter Some astrocytes were very large The optic chiasma contained swollen axons (Fig 1 lower) and was partially demyelinated and gliotic Some leptomeningeal vessels were collagenous and surrounded by lymphocytes

Lesion 2 25 months 20 000 rad The lesion which was situated in the thalamus showed a cavity with necrotic material and calcium concretions attached to its wall (Fig 2 upper) Capillaries in the tissue surrounding the cavity showed endothelial thickening and perivascular cuffing Giant cells (presumably abnormal glial cells) occurred at the edge of the lesion (Fig 2 lower) Proliferation of astrocytes was seen in the wall of the cavity Some proliferat

Table
Physical parameters of irradiation

Lesion No	Time of irradi (min)	Field size (mm ²)	Number of fields	Number of planes	Max angle (°)	Real centre dose (rad)	Killed at (day after irradi)
1	115	7×2	21	2	20	20 000	573
2	59	7×2	21	2	20	20 000	757
3	61	7×2	21	2	20	20 000	781
4	119	7×2	21	2	20	20 000	845
5	45	10×3	21	2	20	15 000	1 110
6	47	7×2	21	2	20	20 000	1 453

angular coordinates, real centre dose (cf LENSEN et coll 1960), and time of irradiation are given in the Table above.

Animal care The animals were kept for study under ordinary farming conditions. During May to October they stayed out of doors in an enclosed well drained pasture with free access to water and salt. During November to April they were kept in a shed on straw. In the latter period they were given hay, salt and water freely, and in addition about 200 g of oats daily per animal. The animals were regularly observed but no neurologic examinations were made. Animals that died or were killed due to intercurrent disease were excluded from the investigation.

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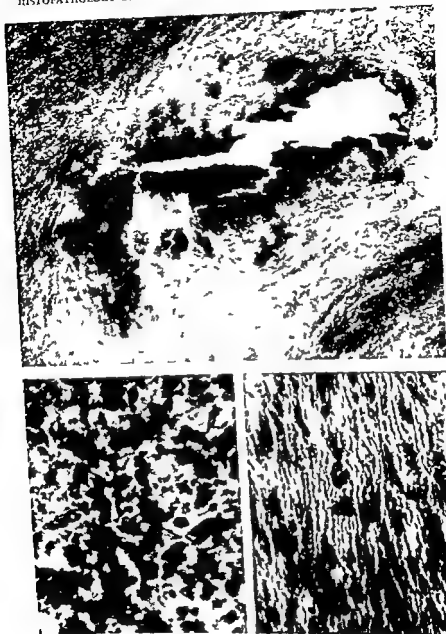


FIG. 3. Lesion 3. 26 months. 20 000 rad. Upper. The lesion lies in the thalamus at the center of a glial scar with deposits of calcium. Densitograph $\times 20$. Lower left. Detail from calcium deposits in upper view. Kossa $\times 200$. Lower right. Pilooid astrocytes at the edge of the lesion. Densitograph $\times 200$.

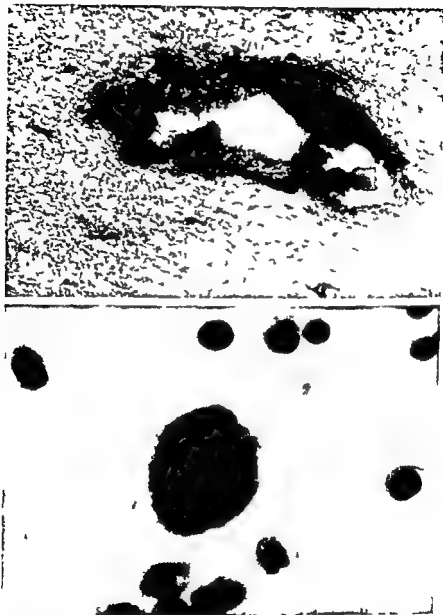


Fig 2 Lesion 2 25 months 20 000 rad *Upper* The lesion lies in the thalamus Deposits of calcium occur in the necrotic tissue and astrocyte proliferation at its edge where perivascular cuffing and round cells also occur Domagk $\times 70$ *Lower* Giant cell with a very large nucleus at the edge of the lesion Domagk $\times 17$

ing astrocytes, occurring adjacent to a band of myelinated fibres just beyond the damaged zone, showed elongated nuclei lying parallel to one another. Nerve cells also occurred close to the lesion. Two very much smaller lesions lay adjacent to the main lesion, both of them containing numerous astrocytes with



Fig 5 Lesion 5 39 months 15 000 rad The lesion lies in the optic tract and consists of a glial scar Adjacent tissue is normal Domagk $\times 8$

several zones of proliferated astrocytes. Some of the astrocytes took an arcuate form and the nuclei were elongated narrow and parallel to one another forming a palisade (piloid astrocytes). None of these cells were in mitosis (Fig 3, lower right). The blood vessels within the irradiated zone had thick collagenous walls and some had a thickened endothelial lining. No evidence of new vessel formation, hemorrhage or teleangiectasis was seen. In the internal capsule and putamen there was a large necrotic zone and wide perivascular changes and astrocyte proliferation.

Lesion 4 28 months 20 000 rad The lesion which was very large, was situated in the pallidum, internal capsule and putamen. Around the necrotic zone there was a reaction on the part of the astrocytes (Fig 4 upper) and the vessels showed perivascular cuffing with small round cells (Fig 4 lower). The lesion extended between fairly well preserved myelinated fibres and collections of nerve cells. Calcium occurred at the edge of the necrosis in a layer of surrounding macrophages.

Lesion 5 39 months 15 000 rad The irradiated zone lay in the optic tract and consisted of numerous astrocyte nuclei and glial fibres lying parallel to one another. Around the gliosed tissue occurred well preserved large nerve cells. Myelinated fibres lay adjacent to the lesion but there was no increase of astrocytes amongst these myelinated fibres. No perivascular cuffing was seen in or around the lesion and there was no increase of vessels, hemorrhage or teleangiectasis (Fig 5).

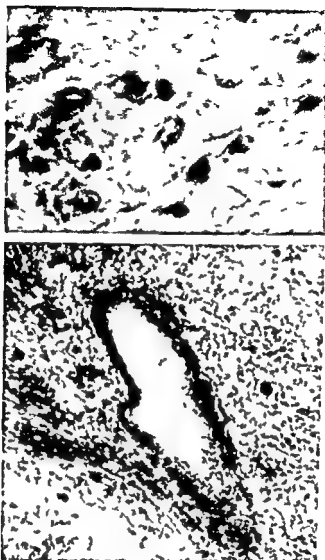


Fig 4 Lesion 4 28 months 20 000 rad Upper Astrocyte proliferation around the necrotic zone Domagk $\times 400$ Lower Vessel showing perivascular cuffing with small round cells from the zone around the necrosis Domagk $\times 120$

loosely arranged fibres and vessels showing perivascular cuffing Occasional giant cells also occurred

Lesion 3 26 months 20 000 rad The irradiated zone situated in the thalamus, consisted of a glial scar made up of astrocytes their fibres and some macrophages Deposits of calcium were present at the edge of the gliosis (Fig 3, upper and lower left) Nerve cells and some calcium deposits occurred among

1 Necrotic stage At a dose level of 20 krad this stage covers approximately the third and fourth weeks after irradiation when necrosis and acute degenerative and inflammatory reactions can be observed. This stage has previously been studied in some detail (cf. LEKSELL et coll. 1960).

2 Stage of resorption This stage is characterized by resorption of cellular debris and beginning glial scar formation. Phagocytic cells are here actively eliminating necrotic debris from the central part of the lesion. Being maximal at the end of the necrotic stage (cf. LEKSELL et coll. 1960) this activity gradually decreases. This stage is here represented by lesions Nos 1, 2, 3 and 4. It is generally characterized by astrocyte proliferation around the necrotic area and occasional giant cells which sometimes have large lobed nuclei. Similar cells have been described by REXED et coll. (1960) and by MONRO & MAIR (1958). This marginal zone also shows a chronic inflammatory reaction with congested vessels and formation of new capillaries, often with endothelial thickening and round cell proliferation.

In the marginal zone of lesions 2 and 3 an isomorphic gliosis with piloid astrocytes was seen. In lesion 3, beginning glial scar formation could be observed. This, however, was not the case in the two months older lesion 4. This might perhaps be explained by the fact that lesion 4 is larger and therefore the resorption phase had not progressed equally far.

3 Late stage This stage seems to be characterized by prominent glial scar formation as demonstrated in Figs 5 and 6 from lesions 5 and 6 respectively. These lesions show no inflammatory reaction, no giant cells, no increase of vessels, no telangiectasis and no hemorrhage. There has been no evidence in this study of cellular elements resembling those of neoplasms in an early stage. Neither have any telangiectasis or bleedings been observed. It may be concluded from the findings presented here that there are no untoward late complications in the goat brain after irradiation of the type described. It should be understood that the material on which this conclusion is based is small and also that the conclusion may be valid only in the species under study.

Since no cellular elements resembling those of neoplasms in an early stage were seen, it gives some support to the belief that the risk of tumour formation may be minor.

SUMMARY

Lesions in the depth of the goat brain were produced by cross-fire irradiation with high energy proton beams and a long term study was performed to determine the late histopathologic effects. No late untoward changes such as cellular elements resembling those of neoplasms in early stage, telangiectasis and hemorrhage were observed.

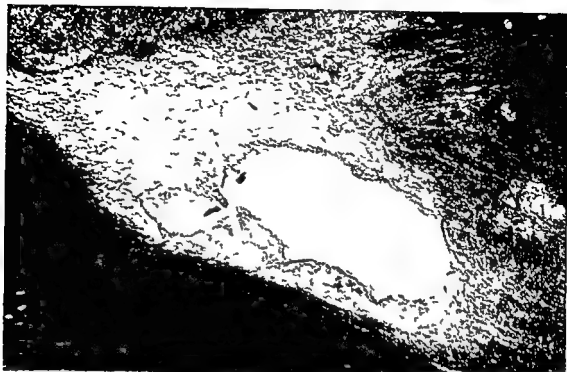


Fig 6 Lesion 6 48 months 20 000 rad The lesion lies in the internal capsule and consists of a cavity surrounded by a glial scar Woelke $\times 10$

Lesion 6 48 months 20 000 rad A well defined ellipsoid lesion was situated in the internal capsule. In the centre there was a glial scar containing a cavity (Fig 6), and around this scar myelinated fibres and nerve cells with an astrocytic reaction. There was no perivascular cuffing and the astrocytes were not of the large size seen in the earlier lesions.

Discussion

The aim of this investigation was to see whether any late untoward reactions occur in animals irradiated by the same technique as used in radiosurgery of the brain.

Pilot studies, in man with lesions made in the thalamus for parkinsonism and in the mesencephalon for pain and for pain or psychiatric disturbances in the frontal lobes are made (LEISLIL 1961, LARSSON et coll 1963, KJELLBERG et coll 1964, and LARSSON 1966). The observations also give a qualitative picture of the characteristics of radio lesions in the brain after irradiation. The phase which follows upon the acute degenerative phase may be divided into three stages.

INSTILLATION OF THIO TEPA (TIFOSYL) IN VESICAL PAPILLOMATOSIS

by

F. EDSVÄR and J. BOMAN

The treatment of vesical papillomatosis is a serious problem in urology. Transurethral excision or coagulation are the conventional methods but the frequency of recurrence is high and repeated treatments are often necessary. Papillomata may sometimes be so widespread inside the bladder that electrocoagulation at one or several sessions may be inadequate as a method of treatment. Attempts have been made to instil radioactive solutions through a rubber bag or directly into the bladder to destroy the papillomata and ^{60}Co , ^{85}Br , ^{199}Au and ^{76}As have been employed to this end (ERIKSSON et coll 1964). The results have not been satisfactory. Recurrence and complications such as contracted bladder, haematuria and vesico-ureteral reflux are not uncommon. High radiation doses are necessary to destroy the papillomata but these may in turn result in damage to surrounding healthy tissue.

The use of chemotherapeutic agents topically in the bladder has long been employed in cases of widespread vesical papillomatosis. The various chemotherapeutic agents injected have included silver nitrate (HERRING 1903), podophyllin (SEMPLE 1948, KELLY & HARTWELL 1954) and over the last ten years thiofosl (thio tepla) (JONES & SWINNEY 1961, ESQUIVEL et coll 1965, VEENEMA

Submitted for publication 18 December 1969

ZUSAMMENFASSUNG

Lesions in der Tiefe von Ziegengehirnen wurden durch Kreuzfeuer Bestrahlung mit Hochenergie Protonenstrahlen hervorgerufen und eine Langzeitstudie um die späten histopathologischen Veränderungen festzustellen wurde durchgeführt. Keine ungünstigen späten Veränderungen wie Zellkernelemente ähnlich denen bei Neoplasmen in frühen Stadien Teleangiectasien oder Blutung wurden beobachtet.

RÉSUMÉ

Les auteurs ont utilisé une irradiation à feux croisés par des faisceaux de protons de haute énergie pour produire des lésions dans la profondeur du cerveau de la chèvre et ont fait des études à long terme pour déterminer les effets histopathologiques éloignés de cette irradiation. Ils n'ont pas trouvé de modifications défavorables tels que des éléments cellulaires ressemblant à ceux des néoplasmes au stade précoce des téléangiectasies et des hémorragies.

REFERENCES

- KJELLBERG R N, KORHNER A M, PRESTON W M and SWEET W H. Intracranial lesions made by the Bragg peak of a proton beam. *In* Response of the nervous system to ionizing radiation p 36. Edited by I J Haley and R S Snider. Little Brown and Company, Boston 1964.
- LARSSON B, LEFSSILL L and RYDÉD B. The use of high energy protons for cerebral surgery. *in* *Acta chir scand* 125 (1963) 1.
- — — et coll. The high energy proton beam as a neurosurgical tool. *Nature* 182 (1958) 1222.
- LEFSSILL L. Cerebral radiosurgery. Presented at the 2nd Int Congr Neurol Surg Washington 1961. *Excerpta medica International Congress Series* No 36 p 5.
- Some principles and technical aspects of stereotaxic surgery. *In* *Pain* p 493. Edited by R S Knighton and P R Dumble. Little Brown and Company, Boston 1966.
- LARSSON B, ANDERSSON B et coll. Lesions in the depth of the brain produced by a beam of high energy protons. *Acta radiol* 54 (1960) 251.
- MAIR W, RYDÉD B and SOUFANDER P. Histology of the surgical radiolesion in the human brain as produced by high energy protons. *Radiat Res* (1967) Suppl No 7 p 384.
- MONRO PAULINE and MAIR W. Radiation effects on the human central nervous system 14 weeks after x radiation. *Acta neuropath* 11 (1968) 267.
- RYDÉD B, MAIR W, SOUFANDER P et coll. Effect of high energy protons on the brain of the rabbit. *Acta radiol* 53 (1960) 289.

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The use of chemotherapeutic agents topically in the bladder has long been employed in cases of widespread vesical papillomatosis. The various chemotherapeutic agents injected have included silver nitrate (HERRING 1903), podophyllin (SEMPLE 1948, KEIL & HARTWELL 1954) and over the last ten years thiofosl (Thio-tepa) (JONES & SWINNEY 1961, ESQUIVEL *et coll* 1965, VIKENMA

Table

Summary of the results of tifosyl treatments reported in the literature

Authors and year of publication	Number of patients	Results	Papilloma	Carcinoma stages			
				A	B	C	D
JONES & SWINNEY 1961	16	No effect	2	—	—	2	—
		Partial destruction	7	—	—	1	—
		Complete destruction	4	—	—	—	—
VERNEMA et coll 1962	57	No effect	11	—	3*	—	—
		Partial destruction	14	—	7*	—	—
		Complete destruction	13	—	—	—	—
ESQUIVEL MACKENZIE & WHITENORE 1964	20	No effect	3	—	6	1	—
		Partial destruction	2	2	2	1	—
		Complete destruction	3	—	—	—	—

* includes those who received prior radiotherapy

et coll 1962) The results of tifosyl treatment reported in the literature are summarized in the accompanying Table

We have used tifosyl instillation since 1963 but since 1967 in accordance with special principles and unitary doses. Twenty nine patients with widespread papillomatosis have had installations of tifosyl (Tio tepa) into the bladder. Eight of these were women and twenty one were men between 46 and 80 years of age. The average period of time for the papillomatosis before tifosyl treatment was 5.3 (1 to 17) years and repeated coagulations had been performed for recurrence.

Method A complete series of treatments consisted of six installations of tifosyl in doses of 50 mg per instillation. Treatment was given on alternate days usually in the outpatient department. The patients assumed several positions during the treatment in order to ensure complete contact between the bladder wall and the active agent. The patients were asked to keep the solution (50 ml) in the bladder for two hours. To avoid dilution of the solution in the bladder, the fluid intake was restricted 12 hours before treatment.

Results

A clinical grading of the effect of treatment on the papillomata was carried out during the cystoscopies which were performed 1 to 6 weeks after the tifosyl treatment. A further series of treatments was usually given if no effect was observed after the first one. No further treatment was given if regression of the papillomata had occurred. No coagulation of the remaining papillomata was performed until it was felt that no further regression could be expected.

The clinical cystoscopic results were codified 0 to 3 where zero indicates no effect or progress, 1 indicates reduction of necrosis of the papillomata, 2 stands for one or more papillomata gone and 3 for all papillomata gone. The following results were obtained:

	Codes	0	1	2	3	Total
One series		4	—	4	6	14
Two series		1	1	3	4	9
Three series		—	—	4	2	6

Complete regression occurred in twelve out of twenty nine cases (41%) even of these occurring directly and five others after coagulation of some small residual papillomata. Complete regression or almost complete regression (codes 2 and 3) occurred in twenty three out of the twenty nine cases (80%). No effect could be observed in five (17%) of the cases.

The number of treatment series in the different groups are given below:

	Number of patients	Series given
Code 0	4	1
	2	2
Code 1	1	2
	4	1
Code 2	3	1
	4	3
Code 3	6	1
	4	2
	2	3

The observation period starting with the first treatment with tifosyl averaged one year (between 2 months and 5 years). The period is too short to allow evaluation of the long term frequency of recurrence. It seems however as if a complete series of tifosyl treatments makes it easier to control the remaining papillomata by coagulation.

Results related to the primary papilloma size In principle, only those patients were chosen for tifosyl therapy who at cystoscopy had thin, slender papillomata spread over the mucous membrane of the bladder at the sites of multiple previous electrocoagulations. There had been much difficulty in effectively destroying new recurrent papillomata by electrocoagulation, and instillation therapy with tifosyl had therefore been adopted. The results are tabulated below according to the size of the lesion.

	Codes	0	1	2	3	Total
'Carpet' (widespread papillomatosis)	4	—	8	9	21	
< Per size	—	1	2	3	6	
= Per size	—	—	1	—	1	
> Per size	1	—	—	—	1	

Results related to the histologic grade of malignancy Biopsies were performed in all cases (except one) before treatment, where the histologic malignancy was grade I or II, as shown below.

	Codes	0	1	2	3	Total
Grade MI	2	1	7	6	16	
Grade MII	3	—	4	5	12	
Grade 0	—	—	—	1	1	

There was no difference in the results of treatment between the two grades of malignancy.

Results related to bladder infection An adequate bacteriologic investigation was not made in connection with the treatment in twenty-one cases in which the results could only be judged by the clinical condition of the patient. Only one of the other eight cases developed infection of the bladder after instillation of tifosyl and this patient experienced no discomfort. The others either had no trouble with constriction or infection, or it was slight. Prophylactic antibiotic therapy was used in connection with the treatment, as related below.

	Codes	0	1	2	3	Total
Infection before and after treatment		1	—	2	1	4
Infection before treatment only	—	1	—	—	—	1
Infection after treatment only	—	—	—	1	—	1
No infection	1	—	—	1	—	2
Not investigated	3	1	8	9	—	21

Complications Four of the twenty nine patients treated with tifosyl had complications that might be considered as side effects. In one man the tifosyl treatment was interrupted after five instillations because of pain and discomfort in the bladder. One woman became tired and had small haemorrhages in the skin of the lower parts of the legs and experienced giddiness three weeks after a tifosyl instillation series: the lowest thrombocyte value was 85 000; all the symptoms disappeared later. The third patient was a man who had a decrease in the thrombocyte value from 186 000 to 47 000 without symptoms or signs in connection with the treatment series: the values returned to normal later. The fourth patient was a woman aged 71, who had had suprapubic cystotomy seven years earlier with resection of a walnut sized papilloma. Five years later the bladder was covered with papillomata. Eight coagulations were performed; one of them in connection with the *ectio alta*. One year before the instillation of tifosyl perforation of the bladder occurred in connection with coagulation and biopsy of the papillomata. Dilatation of the left ureter was present and nephroureterectomy revealed a papilloma in the ureter; postoperative ileus required laparotomy. Multiple papillomata of the bladder occurred 6 months later. Coagulation of as many papillomata as possible was carried out and tifosyl was instilled ten times in doses of 50 mg per instillation every second day. The day after the last instillation the white blood cell count was 9 000 and the thrombocyte count normal. Four days later the white blood cells decreased from 2 500 to 50 for a few days and the thrombocytes from 10 000 to 3 000. The patient died of sepsis probably caused by pancytopenia. Autopsy revealed no tumour or papillomatous tissue in the bladder but marked cystitis with ulceration. The period between the coagulation and the instillation of tifosyl was probably too short and the drug was carried into the blood stream via the ulcerated vesical mucous membrane.

SUMMARY

Twenty nine cases of widespread vesical papillomatosis were treated by the instillation of tifosyl. The method is described. Complete or almost complete regression was achieved in twenty three of the cases.

ZUSAMMENFASSUNG

Spülungen mit Tifosyl zur Behandlung ausgebreiteter Papillomatose der Harnblase wurden an neunundzwanzig Fällen verandt. Die Methode wird beschrieben. In dreißig Fällen erfolgte komplette Heilung oder wenigstens ein beinahe vollständiger Rückgang der Erkrankung.

RÉSUMÉ

Trente neuf cas de papillomatose vésicale étendue ont été traités par instillation de thioseptine. Description de la méthode. On a observé une régression complète ou presque complète dans vingt trois de ces cas.

REFERENCES

- ELIHOORN J., HULTBERG S. and NILSON A. E. Treatment of papillomatosis of the bladder with radioactive arsenic ^{76}As . *Acta radiol Ther Phys Biol* 2 (1964) 1.
- ESQUIVEL E., MACKENZIE A. and WHITMORE W. Treatment of bladder tumors by instillation of thio septa actinomycin D or 5 fluorouracil. *Invest Urol* 2 (1965) 381.
- HERRING H. T. The treatment of vesical papilloma by injections (silver nitrate). *Brit med J* 2 (1903) 1398.
- JONES H. C. and SWINNEY J. Thio septa in the treatment of tumours of the bladder. *Lancet* 1961 II p. 615.
- KELLA M. G. and HARTWELL J. L. Biological effects and chemical composition of podophyllin. *J nat Cancer Inst* 14 (1954) 967.
- SEMPLE J. R. Papillomata of bladder treated with podophyllin. Preliminary report. *Brit med J* 1 (1948) 1235.
- VEENEMA R. J., DEAN JR A. L., ROBERTS M. et coll. Bladder carcinoma treated by direct instillation of thio septa. *J Urol* 88 (1962) 60.

CARCINOMA OF THE LARYNX

I Treatment mainly by primary irradiation

by

KARSTEN JØRGENSEN

Carcinoma of the larynx was treated almost exclusively by surgery in the early part of this century and then only to a limited extent. In the 1920's COUTARD (1932) developed fractionated roentgen irradiation for carcinoma of the pharynx and larynx. This method, adopted in Denmark and modified by JENS NIELSEN (1942), has opened up a possibility of curative treatment of laryngeal carcinoma by primary radiotherapy. The procedure has gradually gained ground in a number of European centres while in U.S.A. the condition is still treated mainly by primary surgery.

The present report deals with the results obtained chiefly by primary radiotherapy, sometimes supplemented by surgery. These results will be compared with those from centres where surgery is mostly employed.

Material A total of 263 patients was examined and treated for carcinoma of the larynx during the period 1942–1963. The review runs to the middle of 1963 when ^{60}Co kilocurie therapy began to be used. It covers only 248 of

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Table 1

Number of cases (total 248) treated for carcinoma of the larynx during the separate 5-year periods from 1 January 1944 up to 1 August 1963 and the crude 5-year survival rates

	Number of cases	Crude 5 year survival rates
1 January 1944—1 January 1949	27	44 %
1 January 1949—1 January 1954	46	65
1 January 1954—1 January 1959	69	67 %
1 January 1959—1 August 1963	106	65 %
1 January 1944—1 August 1963	248	63 %

the patients, fifteen having been excluded because of recurrence following treatment elsewhere (five), histologic examination not performed (five), doubt about the diagnosis on review of the histologic specimens (three), transferred for treatment elsewhere (two)

A few of the 248 patients received minor, palliative doses of radiation and in others radiotherapy was abandoned after some days because of senile debility, general weakness and similar reasons. Apart from the exclusion of the fifteen patients listed above, the series may be considered unselected. The follow up was 100 %.

As to the incidence, CLFMESEN (1965) has reported that carcinoma of the larynx made up 0.91 % of all the cases of carcinoma notified in Denmark from 1943 to 1957. From 1943 to 1948 about 35 new cases were notified annually and from 1953 to 1957 about 60 cases. This increase is considerably in excess of what might have been expected from the increase in the total number of carcinoma cases, so that the incidence of laryngeal carcinoma must be augmenting. Indeed, this is reflected in the present series in which there was a appreciable increase in the number of new cases during the separate 5 year periods (Table 1).

As regards the sex ratio and the age distribution in the present series, 11 % (27/248) of the patients were females and 89 % (221/248) were males. The average age at the time of the histologic diagnosis was 61 years 5 months, as indicated in Fig. 1. The youngest patient was a woman of 27, the oldest a man of 87.

The histologic type was squamous cell carcinoma in 247 cases while one was described as a case of adenocarcinoma, possibly adenomyoepithelioma. Seven of the 247 squamous cell carcinomas were of the stage carcinoma in situ.

The symptoms and signs consisted of hoarseness as initial symptom in 86 % in 9 % there was irritation of the throat, at times with pain radiating to the ear. In the remaining 5 % of the cases, the initial symptoms and signs were equally

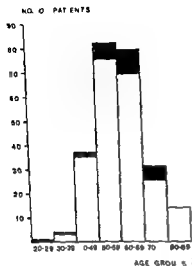


Fig 1 Age distribution at the time of the histologic diagnosis: black fields indicating the number and ages of the women and the white columns those of the men in the series

distributed between dysphagia enlargement of the cervical lymph nodes cough and dyspnoea. The interval from the onset of the initial symptom until the histologic diagnosis was less than 4 months in 41 % 4 to 8 months in 28 % and more than 8 months in 31 % of cases.

Classification This was made according to the TNM system in the UICC modification. The rules of classification are apparent from the following survey (UICC 1968) of the group T Primary tumour

Supraglottic

Glottic

Subglottic

T1—Tumour confined to one anatomical site within the larynx

Tumour confined to laryngeal surface of epiglottis or to an aryepiglottic fold or to a ventricular cavity or a ventricular band

Tumour confined to one vocal cord and mobility of cord remains normal

Tumour limited to one side of the subglottic region exclusive of the undersurface of cord

T2—Tumour confined to one anatomical region within the larynx

Tumour involving the epiglottis extending to the ventricular cavities or bands

Tumour involving both cords with normal mobility of one or both cords with fixation of cord(s)

Tumour extending to two sides of subglottic region exclusive to the undersurface of cords

T3—Tumour extending beyond one anatomical region within the larynx

Tumour of the epiglottis and/or ventricles or ventricular bands and extending into the cords	Tumour extending from cords either to subglottic region or to supraglottic region i.e. to ventricular bands or ventricles	Tumour involving subglottic region and extending on to the cords
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T4—Tumour extending beyond the larynx

Tumour as in T1 T2 or T3 but with direct extension to piriform sinus post cricoid region vallecula or base of tongue	Tumour as in T1 T2 or T3 but with direct extension through cartilage to the piriform sinus or to the postcricoid region	Tumour as in T1 T2 or T3 but with direct extension to trachea skin or post cricoid region
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N — Lymph nodes

N0 — No lymph nodes palpable

N1 — Movable homolateral nodes

N2 — Movable contralateral or bilateral nodes

N3 — Fixed nodes

M — Distant metastases

M0 — No evidence of distant metastases

M1 — Distant metastases present

The distribution of the lesions by site and extent is apparent from Table 2. To restrict the number of groups, cases having presumed lymph node metastases were divided into three groups, a supraglottic, a glottic, and a subglottic group, regardless of whether these were T1, T2, or T4 cases. It will be seen that only 22 cases (9 %) had presumed lymph node metastases in the neck on admission. The incidence of lymph node metastases in the three main groups was as follows: supraglottic seventeen cases (27 %), glottic four (2 %), and subglottic one case (8 %).

Method of treatment A total of 217 cases were treated primarily by irradiation and 29 primarily by surgery. Conventional roentgen radiation was used throughout the period 1944—1963. From the beginning of 1959 up till August 1963 most cases were treated by a ^{60}Co unit whose radiation source was of decicurie strength, so that during the last 5 year period the irradiation was administered by two different methods. The small field size of the ^{60}Co unit made it suitable for treating fairly small neoplasms while the conventional roentgen apparatus was employed mainly for larger growths and lymph node metastases.

Table 2

Site of 248 cases of carcinoma of the larynx grouped according to the TNM system (UICC)

	Supra glottic	Glottic	Sub- glottic	Total
T1\0\0	8	81	—	89
T2\0\0	9	32	—	41
T3\0\0	21	53	9	83
T4\0\0	7	3	3	13
T1+2+3+4				
N1+0+3\0	17	4	1	22
Total	67	173	13	248

Conventional roentgen radiation was administered to two opposed fields, one field being treated each day. An extra field was added anteriorly however if necessitated by a large growth. The field size was usually 6 cm \times 6 cm but was extended when indicated by the size of the neoplasm or by lymph node metastases. The tumour dose was generally about 6 000 R over a total treatment period of 30 to 35 days up till 1950 the dose was somewhat larger and the period longer.

A ^{60}Co unit of 10 to 15 decacurie was generally used during the period 1959—1963 (maximum field size 3 cm \times 3 cm FSD 10 cm). Small growths of the vocal cords were irradiated to two anterolateral fields using a wedge filter with its base medially. The calculated tumour doses were usually 5 000 to 7 000 R and the treatment period 25 to 35 days.

Two of the twenty nine cases treated primarily by surgery had endoscopic removal of very small carcinomas of the vocal cords. Apart from these two cases the indications for primary surgery were large extension of the tumour, a large mass causing respiratory obstruction or a growth invading or penetrating the laryngeal cartilage. Involvement of the hypopharynx or subglottic location in cases of not too advanced age and in good general condition were also indications for primary surgery (only total laryngectomy). All the operations were performed by the same surgeon (H. C. Andersen).

Follow up. All the patients were examined by the radiotherapists and otolaryngologists at weekly conferences. After completed irradiation the subjects attended as out patients first at intervals of 2 to 3 months. Any suggestion of residues at the termination of radiotherapy resulted in the patients being admitted about 6 to 8 weeks later for examination by direct laryngoscopy and

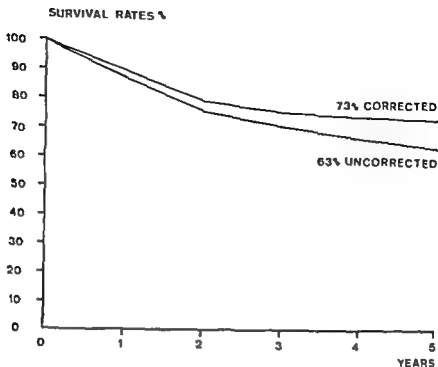


Fig. 2 Decrement curves for 248 patients with carcinoma of the larynx. The upper curve is corrected for mortality from other causes; the lower curve is uncorrected.

biopsy. A positive biopsy usually meant that the patient was subjected to operation (a few refused and some were too weak). Patients without evidence of residues or recurrence were followed for 10 years, but after the first 3 years were seen only once a year.

Results

The 5 year survival rates for all the patients are listed in Table 1 and divided into 5 year periods. The results have been almost stationary for 15 years.

Fig. 2 illustrates the total result depicted in two decrement curves. The lower, uncorrected curve indicates the calculation of the total mortality coefficients for each individual year. The upper curve is produced by the calculation of the corrected mortality coefficients for the separate years, eliminating other causes of death. The latter coefficients were calculated from the formula

$$q_n = \frac{c}{1/2 + c + l}$$

(NOHRMAN 1953), where q_n is the corrected mortality coefficient in the n th year, c the deaths from the condition occurring in the n th year, l the number

Table 3

Subdivision of the 248 cases of carcinoma of the larynx according to the type of primary treatment given

Primary treatment	Number of cases	5 year survival recurrence free	Deaths	Alive with recurrence	Crude 5 year survival rates
Irradiation	219	140	78	1	64
Surgery	29	15	13	1	55
Total	248	155	91	2	63

Table 4

Results of treatment in 248 cases of carcinoma of the larynx subdivided into the three main locations

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
Supraglottic	67	18/3	13/25	2/3	33/67	53
Glottic	173	87/117	26/40	9/16	117/173	68
Subglottic	13	1/3	1/9	5/8	7/13	54
Total	248	101/152	40/67	16/99	157/248	63

IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

of deaths due to other causes and the number of patients alive at the end of the year concerned. The corrected curve thus represents the cancer deaths most of which occurred during the first two years. The curve is almost horizontal after the fourth year there being only a few late deaths from carcinoma.

The types of primary treatment are grouped in Table 3. A comparison of the surgical and radiologic treatments is out of question as the former consisted of only a few relatively advanced cases and was thus a special selected group.

Table 4 gives the results in the three main groups. Neoplasms arising from the vocal cords proper apparently carry the most favourable prognosis.

Supraglottic group Table 5 indicates that the prognosis for T1 + 2 + 3N0 is good. The explanation of the supraglottic group as a whole being in the most unfavourable situation is the relatively high incidence of lymph node metastases and the relatively large number of T4N0M0 cases.

Table 5

Results of treatment in 62 cases of supraglottic carcinoma of the larynx subdivided according to the TNM system (UICC)

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
T1N0M0	8	6/7	1/1	—	7/8	71 %
T2N0M0	9	2/5	3/4	—	5/9	
T3N0M0	21	6/9	8/10	1/2	15/21	
T4N0M0	7	0/3	0/2	1/2	1/7	
T1+2+3+4						25 %
N1+2+3M0	17	4/8	1/8	0/1	5/17	

* IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

Table 6

Results of treatment in 173 cases of glottic carcinoma of the larynx subdivided according to the TNM system (UICC)

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
T1N0M0	81	50/63	14/16	2/2	66/81	81 %
T2N0M0	32	15/24	4/7	1/1	20/32	63 %
T3N0M0	53	17/26	8/17	5/10	30/53	57 %
T4N0M0	3	0/1	—	1/2	1/3	1/3
T3+4 N1+2+3 M0	4	0/3	—	0/1	0/4	0/4

* IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

Glottic group: The results are presented in Table 6 and illustrated by decrement curves in Fig 3 by the same method of calculation as mentioned above. The two top curves in Fig 3 indicate that the T1 group had a relatively high mortality from causes other than malignancy. Seventeen of the thirty two T2 cases had fixed vocal cords and fifteen mobile cords. The latter

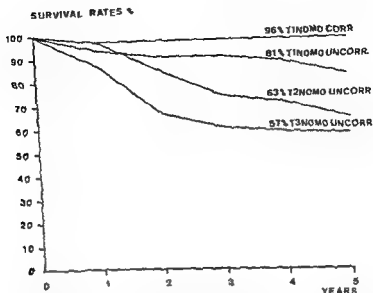


Fig 3 Decrement curves for patients with carcinoma of the glottis. The uppermost curve is corrected for mortality from causes other than carcinoma of the larynx and all the other curves are uncorrected.

category were cases in which the tumour involved both cords but mobility was preserved. The crude 5 year survival rates for these two groups were cases with fixed cords 59% (10/17) cases with mobile cords 71% (10/14). Further mention will be made of this difference later and the results in the T3 group will also be considered in greater detail in the discussion.

The prognosis with fixed cords in the 173 cases of carcinoma of the glottis was assessed. A total of 58 cases had fixed cords and 115 had freely mobile vocal cords. The 5 year results for these two groups were 52% (30/58) and 76% (87/115) respectively.

Subglottic group The results for this group are listed in Table 7. The number of cases was small but the indication seems to be that radiotherapy alone has little chance of effecting a cure.

Comparison of the results of roentgen and ^{60}Co (decacurie unit) radiation It is of considerable interest to compare the 160 to 250 kV apparatus with the ^{60}Co unit of decacurie strength as regards 5 year survivals without other treatment. For this purpose it was decided to compare cases treated primarily by ^{60}Co during the period 1959–1963 with those which received primary

Table 7

Results of treatment in 13 cases of subglottic carcinoma of the larynx subdivided according to the TNM system (UICC)

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURC* for recurrences and res	Primary surgery	Total	
T3N0M0	9	0/2	1/2	4/5	5/9	5/9
T4N0M0	3	1/1	—	1/2	2/3	2/3
T3N1M0	1	—	—	0/1	0/1	0/1

* IRR = irradiation SURC = surgery after failure of irradiation res = residual tumour

roentgen irradiation during the period 1949—1963. A considerably larger number of T4 and N1 + 2 + 3 growths were present in the latter group, and these were therefore excluded from both groups. This leaves only T1, T2 and T3N0M0 cases. Thus curtailed, the groups are fully comparable as regards classification, sex ratio, and age distribution. The difference in time will be disregarded as it is probably of no great importance.

A total of 70 cases treated with the ^{60}Co decacurie unit are compared in Table 8 with 97 cases treated by the conventional roentgen apparatus. A striking difference was apparent between these two groups in the cure rates after irradiation as the only treatment. Indeed, statistical calculation disclosed that the difference was distinctly significant. It is apparent from the table that there was but little difference between the crude 5 year survival rates in the two groups (71.4 and 74.2 %, respectively), this being due to two factors: (1) a relatively far larger number of the ^{60}Co group had a secondary operation, (2) the surgical results in the ^{60}Co group were considerably better than in the roentgen group. This is because ^{60}Co therapy also failed in a number of cases with small vocal cord carcinoma which have a favourable prognosis after secondary surgery. (Nearly all the partial laryngectomies were in the ^{60}Co group, cf. Table 9.)

The inefficacy of the ^{60}Co unit (decacurie) might seem surprising. There are at least two explanations. The relatively weak radiation source with a focus-skin distance of 10 cm and a field size of 3 cm × 3 cm did not afford an entirely expedient course of the isodose curves. This combined with an inaccurate adjustment technique, is presumably the reason why the calculated tumour doses were not attained in a number of instances.

Table 8

Results in two comparable groups of cases one treated with the ⁶⁰Co unit and the other with the conventional roentgen apparatus

	5 year cure rates after irradiation as only treatment		Percentage and number of cases subjected to sec- ondary surgery		Results of second ary surgery at the 5 year limit		Crude 5 year survival rates	
Co-group	38.6	(27/70)	41.0	(29/70)	79.3	(23/29)	71.4	(50/70)
70 cases								
Roentgen group	58.7	(57/97)	24.7	(24/97)	62.5	(15/24)	74.2	(72/97)
97 patients								
Level of significance	Standard error of the difference 7.48 difference 0.7 times the standard error		Standard error of the difference 5.84 difference 2.5 times the standard error				Standard error of the difference 6.96 difference 0.4 times the standard error	

Results of surgery. A total survey of the surgical procedures as well as the 5 year survivals from the institution of the primary treatment is given in Table 9. Out of 22 partial laryngectomies eight were done because of malignant residues and fourteen for recurrence. At a 5 year limit the survival was 4/8 and 13/14 respectively indicating that partial laryngectomy is less suited for the treatment of residues. During the period under discussion a total of 75 total laryngectomies were carried out: 27 primary and 48 secondary for residues or recurrence. Of these 75 patients forty were alive at the 5 year limit. It must be mentioned that laryngectomy and dissection of cervical lymph nodes were not always performed at the same session. As for the twenty five cervical node dissections the histologic examination revealed metastases in nine and non-malignant changes in thirteen patients. The result was not stated in three patients. All nine patients with positive histologic findings died while all but one of the histologically negative patients survived. The indication for cervical node dissection was palpable and doubtful lymph nodes on admission or in the course of the laryngectomy.

Local quality after radiotherapy and surgery. The quality of the voice was assessed from data in the case records of patients who survived for more than 5 years after irradiation as only treatment. The voice was normal or almost normal in 90% (66/73) of the roentgen irradiated patients. The corresponding values for the ⁶⁰Co group were 82% (23/28). The fourteen patients subjected to partial laryngectomy, all of whom had passed the 5 year limit, were articulate.

Table 9

Operations (a total 103) performed in 97 of 248 patients treated during the years January 1944 — in clusive of July 1963 — 29 primary surgery — 73 for residues or recurrences — 1 perichondritis

	Supra glottic	Glottic	Sub glottic	Total	At 5 year limit	
					Alive	Dead
Endoscopic excision	—	2	—	2	2	—
Laryngofissure with cordectomy	—	1	—	1	1	—
Partial laryngectomy	3	13	—	16	14	2
Partial laryngectomy with laryngectomy later	2	3	1	6	3	3
Total laryngectomy	13*	26	8	47	23	24
Total laryngectomy and neck dissection	10	11	1	22	14	8
Neck dissection alone	3	—	—	3	—	3
Total	31*	56	10	97	57	40

* including the patient operated for perichondritis

although their voices were rough and hoarse. Vocal function in the patients who underwent total laryngectomy has been studied by JENSEN & BALSLEV (1967) who reported that about 60% attained satisfactory oesophageal voices. A few used a voice vibrator.

Side effects of radiotherapy. Acute radiodermatitis of varying degree was observed in all the roentgen irradiated patients, whereas those irradiated with ^{60}Co exhibited only mild cutaneous reactions. Chronic cutaneous changes of a more serious nature did not occur.

All the irradiated patients developed more or less marked epithelitis of the larynx. More severe reactions resulting in oedema were but few, and only two patients required tracheotomy during the treatment, both these had large T3 glottic tumours with a cramped space. Chronic mucosal complaints with dryness and tenderness of the pharynx occurred in seven patients, three of whom, however, reported that these had disappeared after about 5 years.

Perichondritis, presumably induced by irradiation, was observed in five patients, three of those treated by ^{60}Co and two by roentgen irradiation. One patient had to have laryngectomy, two tracheotomy, and two patients had severe symptoms for about 2 years. Apart from these, a few patients had perichondritis due most probably to a recurrence of the neoplasm.

Complications and sequelae of surgery. The complications in 103 operations were few. There were no deaths or serious sequelae during the operation or in

Table 10

Relations between duration of initial symptom stage of disease and rate of survival

Duration of symptoms before histologic diagnosis	0-3 months	4-7 months	8-11 months	12-15 months	16-19 months	More than 20 months	No information
Number of patients	101	69	15	18	17	23	5
Crude 5 year survival rates	65/101	43/69	9/15	10/18	9/17	18/23	3/5
	64	62	60	56	53	78	
Groups T1+2/T1+2+3+4	(64/101)	(40/69)	(21/50)44			9/23	3/5
	63	59					

the first few postoperative weeks. Apart from small suture leaks, fistulas to the hypopharynx of short duration occurred in seven of the totally laryngectomized patients. Chronic fistulas requiring one or more operations, occurred in four patients and several patients had plastic stoma repair.

One of the partially laryngectomized patients had to be fitted with a cannula because of stenosis and one complained of slight stridor on exertion. The special problems of the totally laryngectomized patients such as dyspnoea, discharges and social problems have been thoroughly discussed by JENSEN & BALSLEV.

Prognosis in relation to duration of the initial symptom. As already mentioned there were quite marked differences in the intervals from the onset of the first symptom until the histologic diagnoses. It is of interest to assess the prognostic role of this factor. The entire series was therefore divided into groups according to whether the histologic diagnosis was made 0-4 5-7 8-12 or more months after the onset of the initial symptom (Table 10). The longer the interval the poorer the prognosis.

Table 10 also indicates the staging in relation to the duration of the initial symptom. The total numbers of T1 and T2 cases are given as percentages of the total numbers for the separate interval groups. As might be expected this reveals that a long duration of symptoms produced relatively fewer T1 and T2 cases or in other words a late diagnosis means a considerably larger number of advanced cases (T3 and T4 cases).

The group of cases in which the initial symptom had been present for more than 20 months differs entirely from what might have been expected. The explanation may be that the majority in this group consisted of cases with pre-existing chronic laryngitis although no exact data or values can be given. This group contains a relatively large number of cases classified as advanced. Considering the extremely favourable prognosis it seems justified to conclude that carcinoma

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indicate that these occurred mainly within the first two years. The practical consequences must be that materials analysed in this way afford an accurate expression of the efficacy of treatment in a series provided a reasonable number of cases have been followed for more than 3 years. The special factors applying to the three main groups will now be discussed.

The treatment in the supraglottic group is complicated mainly by the high incidence of lymph node metastases. Dissection of the cervical lymph nodes with histologic evidence of lymph node metastases in nine cases did not lead to any cure whereas four cases with presumed metastases were cured by irradiation. The tendency in recent years in centres using surgery as primary treatment seems to have been cervical node dissection in dealing with supraglottic carcinoma even when no lymph nodes are palpable but paying regard to the extent of the tumour (JACKSON & NORRIS 1962; MCCOMB 1966).

The results within the group of glottic T1 cases are in the same range as reported from elsewhere (MCCABE *et coll* 1960; CHABAZIAN *et coll* 1967; PEREZ 1968). An attitude to treat this group primarily by irradiation appears to exist in most centres.

The glottic T2 cases may be classified as already mentioned, into two subgroups with or without fixation of the vocal cords and these subgroups presented a distinct prognostic difference (59 % and 71 % crude 5 year survival rates respectively). HEISE & BAYLIS, dealing with the same problem reported a similar prognostic difference between the two subgroups 57 % for fixed and 70 % for unfixed cases. On this basis it would seem reasonable to suggest that the T2 group be divided into two subgroups T2 + fix with fixation of cord(s) and T2 — fix without fixation of cord(s). It is tempting to transfer T2 + fix to the T3 group but probably not permissible since all T2 + fix cases cannot be said with certainty to be more extensive or more deeply infiltrating than T2 — fix cases.

Another factor is the prognostic role of fixed vocal cords in all glottic carcinomas considered together. The survival rates for the two groups with fixed and unfixed vocal cords in this series were 52 % and 76 % respectively. This finding is not surprising considering that fixation is presumably usually a sign of more deeply infiltrating processes. The prognostic role of vocal cord fixation has been discussed by SLANE KNUDSEN (1960), RYGDAL & HANSEN (1966) and MARTENSON *et coll* (1967).

Glottic T3 cases from our series appear to be comparable with similar cases treated in other centres where surgery is the primary therapeutic principle. The results are largely the same with crude 5 year survival rates of a little below 60 %. The frequency of total laryngectomy in the surgical materials is about 85 % (SMITH *et coll* 1961) while in our series it was 43 % (23/53). Of the

in cases with chronic laryngitis is to some extent erroneously placed in an advanced group

Discussion

Investigation of the symptoms revealed that the initial symptom of laryngeal carcinoma was hoarseness in 86 % of cases. By way of comparison, it may be mentioned that in a series of 1498 cases BALZELI & PUTNEY (1954) gave this percentage as 85.5. In more than half the number of our cases the diagnosis was reached more than 4 months after the onset of the initial symptom. Table 10 indicates that a late diagnosis makes for a poorer prognosis and a more advanced stage of the disease. It may be concluded therefore that the prognosis may still be improved by earlier diagnosis, considering the excellent prognosis in the less advanced cases.

The TNM system used in the analysis proved to be most useful, in accordance with the findings of others (SMITH et coll 1961, MORRISON & DEELEY 1962, JOHNSON & SISSON 1964, MCNELIS 1964, ALEXANDER CASSADY 1966, CAULK 1966, HEISE & BAYLIS 1966, RYGDAL & HANSEN 1966, BACLESSE 1967, MARTENSON et coll 1967, SPENCER 1967, and TASKINEN 1969). The possibility of comparing the different series and of discussing the way of treatment has been greatly improved.

The total result of a 63 % crude 5 year survival rate in our series is high compared with others published in recent years. However, our series comprises mainly growths that were relatively moderately advanced, there being, in particular, but few T4 and N1 + 2 + 3 cases compared with other series. These cases made up 14 % of the entire series. Other materials, all of which are American except that of MARTENSON et coll (1967), have been of the following composition (the percentages stated represent the number of T4 and N1 + 2 + 3 cases in relation to the total cases)

ALEXANDER	~ 20 %	~ 60/306 cases
HEISE & BAYLIS	29 %	226/788
JOHNSON & SISSON	29 %	29/100
MCNELIS	42 %	39/93
MARTENSON et coll	15 %	87/578
SMITH et coll	33 %	201/600
Present series	14 %	35/248

None of the series appears to have been selected except the series of JOHNSON & SISSON and of MCNELIS. It is thus evident that our series contained a relatively small number of advanced cases and a relatively large number of small growths. The decrement curves (Figs 2 and 3) representing the deaths from carcinoma

indicate that these occurred mainly within the first two years. The practical consequences must be that materials analysed in this way afford an accurate expression of the efficacy of treatment in a series provided a reasonable number of cases have been followed for more than 3 years. The special factors applying to the three main groups will now be discussed.

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Glottic T3 cases from our series appear to be comparable with similar cases treated in other centres where surgery is the primary therapeutic principle. The results are largely the same with crude 5 year survival rates of a little below 60 %. The frequency of total laryngectomy in the surgical materials is about 80 % (SMITH *et coll* 1961) while in our series it was 43 % (23/53). Of the

5 year survivors 30 % (9/30) had undergone total laryngectomy. Apart from that, a few cases in the T3 group (four in all) had had partial laryngectomy.

The analysis of the glottic T3 group thus displayed the advantages of primary radiotherapy possibly followed by secondary surgery. The survival rates are equal to those after primary surgery but the percentage of cases with preserved laryngeal function is about twice to three times higher than after primary surgery.

The subglottic group comprised 13 cases. Radiotherapy alone appears unsuitable for curing carcinoma in this location, whereas surgery, with or without irradiation, affords essentially better results, as suggested by FALBE HANSEN (1955) and MARTENSON *et coll* (1967). In advanced cases of subglottic carcinoma, meaning almost invariably carcinoma that has invaded the vocal cords and fixed them, the treatment of choice is perhaps complete radiotherapy followed by total laryngectomy and possibly dissection of cervical lymph nodes. This rigorous procedure may be considered reasonable as there is a great risk of fatal delay in the detection of malignant residues or recurrence. Possibly, it is also preferable in advanced laryngeal carcinoma in sites other than the subglottic, a view held by JOHNSON & SISON (1964). GOLDMAN *et coll* (1968) have used this combined treatment in eight T4N0M0 cases, with a 100 % survival so far for 1 to 6 years.

Conclusion

The results of treating laryngeal carcinoma mainly by primary irradiation must, in keeping with previous findings, be acceptable (NIELSEN 1954, CANTRIL 1960, TUDWAY & FREUNDLICH 1960, LEDEPMAN 1961, WILSON 1961, BRICE 1963 *et coll*, WANG & SCHULTZ 1963, ORMEROD 1964, BOZZI *et coll* 1966, JOLLES 1966, RICARD & HANSEN 1966).

This therapeutic method affords results equal to those of primary surgery but unlike the latter possesses the important advantage of considerably increasing the number of patients with preserved laryngeal function. Surgical series from recent years with excellent survival rates have had a laryngectomy frequency of 60 to 75 % (SMITH *et coll* 1961, JOHNSON & SISON 1964, McNELIS 1965, ALEXANDER & CASSIDY 1966, SPENCER 1967). By way of comparison it may be mentioned that the frequency in our series was 30 % (75/248). It must be borne in mind however that the series named in this comparison contained a relatively larger number of advanced cases. Not uncommonly the view is maintained that the patient's chances of survival are reduced when radiotherapy rather than surgery is the primary treatment. This view has been disproved by our results. Another argument against primary radiotherapy has been that it prevents the possibility of subsequent supplementary minor surgical procedures (partial

laryngectomy). However it is apparent from the present results (Table 9) as well as those of others (FALBE-HANSEN & SÆVAGE-KNUDSEN 1964) that partial laryngectomy may prove an excellent operation in spite of preceding irradiation.

The safety of irradiation as the primary treatment is clearly apparent from the analysis presented in Table 8. A failure in radiotherapy irrespective of the cause does not necessarily affect the end result. An effective follow up with early diagnosis of malignant residues or recurrence followed by secondary surgery, may bring the result almost up to the level where radiotherapy is at its best although of course at the cost of laryngeal function.

SUMMARY

A total of 248 patients with carcinoma of the larynx were treated during the period 1944—1963: 219 by primary irradiation and 29 by surgery. The crude 5 year survival rate for the entire series was 63 per cent. The 5 year survival rates with radiation therapy were largely the same as those obtained by surgery. The number of patients with preserved laryngeal function was however twice to three times greater after treatment mainly by primary irradiation.

ZUSAMMENFASSUNG

Insgesamt wurden 248 Patienten mit Kehlkopfkarcinom während den Jahren 1944—1963 behandelt: 219 Patienten wurden primär mit Bestrahlung behandelt und 29 Patienten wurden operiert. Nach 5 Jahren waren 63 Prozent der Patienten am Leben. Die 5 jährige Überlebensrate für die bestrahlten und die operierten Patienten war praktisch gleich. Die Anzahl von Patienten mit erhaltener Kehlkopffunktion war jedoch zwei bis dreimal grösser nach Behandlung hauptsächlich mit Primärbestrahlung.

RÉSUMÉ

Un total de 248 malades ont été traités entre 1944 et 1963 pour cancer du larynx. 219 malades ont été traités par irradiation primitive et 29 par chirurgie. Le taux brut de survie à 5 ans pour la série entière est de 63 pour cent. Le taux de survie à 5 ans après radiothérapie est en gros le même qu'après chirurgie. Cependant le nombre de malades qui avaient conservé la fonction laryngée était deux à trois fois plus élevé après radiothérapie primitive.

REFERENCES

- ALEXANDER F. W. and CASSADY C. L. 306 laryngeal carcinomas. Staging and end results. *Arch. Otolaryng.* 83 (1966) 607.
 BACHELSE I. Comparative study of results with conventional radiotherapy (200 kV) and cobalt therapy in the treatment of cancer of the larynx. *Clin. Radiol.* 18 (1967) 292.
 BALZELL W. H. and PUTNEY F. J. Cancer of the larynx. *Arch. Otolaryng.* 60 (1954) 478.
 BOZZI E., COVA F. L., BOTTI C. and TOSSI G. Surgery possibilities for laryngeal and hypopharyngeal carcinomas after treatment with high-energy betatron electrons. *Acta otolaryng. (Stockh.)* 67 (1966) 44.

- BRYCE D P, IRLAND P E and RIDER W D Experience in the surgical and radiological treatment in 500 cases of carcinoma of the larynx *Ann Otol* (St Louis) 72 (1963) 416
- CANTRIL S T Radiation therapy in cancer of the larynx *Amer J Roentgenol* 83 (1960) 17
- CAULK R M End results of radiotherapy in laryngeal cancer based upon clinical staging by the TNM system *Amer J Roentgenol* 96 (1966) 588
- CHABAZIAN C M and REGATO J A Cobalt 60 teletherapy of early carcinoma of the vocal cords *Amer J Roentgenol* 99 (1967) 333
- GLENNESBY J Statistical studies in the aetiology of malignant neoplasma *Acta path microbiol scand* (1965) Suppl No 174 Part 2 p 6
- COUTART H Roentgen therapy of epitheliomas of the tonsillar region, hypopharynx and larynx from 1920 to 1926 *Amer J Roentgenol* 28 (1932) 133
- DANIL O, JACOBSSON F and WALSTAM R Telegamma therapy of laryngeal carcinoma *Acta Un int Cancer* 20 (1964) 1735
- FALBE HANSEN J Results of surgery in the treatment of laryngeal cancer *J Laryng* 69 (1955) 255
- and SVANE KNUDSEN V Partial laryngectomy for laryngeal cancer Five year results *J Laryng* 78 (1964) 1
- GOLDMAN J L, FRIEDMAN W H and BLOOM H S Elective neck dissection in laryngeal and laryngopharyngeal cancer *Laryngoscope* 78 (1968) 539
- HEISE H and BAYLIS P Clinical staging in cancer of the larynx a report on 788 cases using the American Joint Committee's Method of Stage Classification *J nat Cancer Inst* 36 (1966) 45
- JACKSON C L and NORRIS C M Cancer of the larynx IV Section of treatment anatomic considerations and criteria *CA (N.Y.)* 12 (1962) 149
- JACOBSSON F Telerradium treatment of laryngeal carcinoma at Radiumhemmet Stockholm *Acta radiol* 38 (1952) 143
- Radiotherapy of carcinoma of the larynx *Acta otolaryng* (1954) Suppl No 116 p 153
- JENSEN T S and BALSLEV A 104 cases of total laryngectomy *Nord Med* 78 (1967) 1057
- JOHNSON N E and Sisson G A Carcinoma of the larynx Review of 100 cases *Laryngoscope* 74 (1964) 710
- JOLLES H Long term results of treatment of cancer of the larynx *Clin Radiol* 17 (1966) 71
- LEDERMAN M Place of radiotherapy in treatment of cancer of the larynx *Brit Med J* 1961 i p 1639
- MCCABE H F and MAGIELSKI J F Carcinoma of the larynx comparative experience in the management of 951 limited lesions *Ann Otol* (St Louis) 68 (1960) 1013
- MCCOMB W S Cancer of the larynx *Cancer* 19 (1966) 149
- MGNELIS F L Laryngeal carcinoma classified by clinical staging *Arch Otolaryng* 82 (1964) 173
- MÄRTENSON B, FLULR E and JACOBSSON F Aspects on treatment of cancer of the larynx *Ann Otol* (St Louis) 76 (1967) 313
- MORRISON R and DEELEY T J The treatment of carcinoma of the larynx by supervoltage radiotherapy *Clin Radiol* 13 (1962) 145
- NIELSEN J Rational association of irradiation and surgery in the treatment of laryngeal carcinoma *J Laryng* 68 (1954) 370
- and STRANDERØ O Roentgen treatment in cancer of the larynx *Acta radiol* 23 (1942) 189 and 255
- NOHRMAN B A Survival rate calculation *Acta radiol* 39 (1953) 78
- ORMEROD F C Carcinoma of the larynx *Rep Inst Laryng Otol* 15 (1964/65) 1

- PEREZ C A HOLTZ S OGURA J H et coll Radiation therapy of early carcinoma of the true vocal cords *Cancer* 21 (1968) 764
- RYGÅRD J and HANSEN H S Five year symptom free survival rate of 414 consecutive cases of laryngeal cancer referred to the Radrum Centre Copenhagen *Acta oto laryng* (Stockh) (1966) Suppl No 224 p 482
- SMITH R R CAULK R M RUSSELL W O and JACKSON C L End results in 600 laryngeal cancers using the American Joint Committee's Proposed Method of Stage Classification and End Results Reporting *Surg Gynec Obstet* 113 (1961) 435
- SPENCER J T Cancer of the larynx *Laryngoscope* 77 (1967) 962
- SVANE KNUDSEN V Cancer laryngis Munksgaard Copenhagen 1960
- TASKINEN P J Radiotherapy and TNM classification of cancer of the larynx *Acta radiol* (1969) Suppl No 287
- TNM Classification of malignant tumours International Union against Cancer Geneva 1968
- TUDWAY R C and FREUNDLICH H F The use of a cobalt 60 beam unit for the treatment of carcinoma of the larynx *Brit J Radiol* 33 (1960) 98
- UICC Union Internationale Contre le Cancer Geneva 1968
- WANG C C and SCHULTZ M D Cancer of the larynx Its management by radiation therapy *Radiology* 80 (1963) 963
- WILSON C P Treatment of intrinsic carcinoma of the larynx *J Laryng* 75 (1961) 785

CONCENTRATION OF ZINC IN SOME HARD AND SOFT TISSUES OF RAT DETERMINED BY NEUTRON ACTIVATION ANALYSIS

by

BO BERGMAN

Zinc is an essential element for the organism and is present in most organs and tissues in man and animals in varying quantities. High concentrations have been reported for hard tissues, such as bone (LUTZ 1926, TAYLOR 1961, ALEXANDER & NUSBAUM 1962, SOREMARK & BERGMAN 1952) and teeth (CRUICKSHANK 1936, SAMSANI & SOREMARK 1961, LUNDBERG et coll 1965, BABICKI & TAYLOR 1966, NIXON et coll 1967). Previously reported values of zinc concentration in skeletal tissues have most often been concerned with long bones. High concentrations of zinc have been localized by means of autoradiography in mineralizing areas in long bones (HAUMONT & VINCENT 1961, HAUMONT 1963, KINNAMON 1963).

The present study was initiated in order to compare quantitatively the zinc concentration of the following skeletal tissues of young and adult rats: mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis. To allow for a more complete comparison with previous studies, the teeth and some soft tissues were also included. The results obtained in the present investigation are intended

Table 1

Sensitivity of neutron activation analysis for zinc: a compilation of values reported in the literature

Author(s)	Isotope measured	Neutron fluence cm ² sec	Irradiation time	Sensitivity
MEINKE 1955		5×10^{10}	Saturation	0.04 µg/ml 0.007 µg/ml
LEDDICOTTE et coll 1958	Zn	5×10^{10}	Saturation	0.07 ppm
BOWEN 1959	⁶⁵ Zn	10	12 hours	0.65 ppm
PARR & TAYLOR 1964	Zn	10	7 days	0.01 ppm

to serve as a complement to a scintillation study on the distribution of radiozinc in rats (BERGMAN 1970).

Various methods have been utilized for the quantitative determination of microelements in biologic materials. For the determination of zinc in tissues and body fluids, colorimetric methods have been used extensively (VALLEE & GIBSON 1948; BERENSTAM 1952; WOLFF 1956; MALMSTROM 1956). Spectrographic analysis has been employed by SCOUAR (1939), GRIFFITH et coll (1954), MONAGELLI et coll (1956), BUTT et coll (1964), PFEILSTICKER (1965) and STRAIN et coll (1966) and radiometric titration by LANDGREBE et coll (1968). Roentgen ray fluorescence has been used by ALEXANDER & NUSBAUM (1962) and ZEITZ & LEE (1966) and atomic absorption spectrophotometry by PRASAD et coll (1955), MACAPINLAC et coll (1966), PIHL & GUSTAFSON (1967), HACKLEY et coll (1968) and BOQUIST & LERNMARK (1969).

In recent years neutron activation has come into use more and more for analysis of microelements in biologic tissues. The method has been reviewed and discussed in detail by e.g. BOWEN & GIBBONS (1963), SOREMARK (1965) and BOWEN (1956).

Values reported in the literature on the sensitivity of neutron activation analysis for zinc are compiled in Table 1. The sensitivity will be further increased by radiochemical separation of the radioactive zinc present in the sample (SAMSAHL 1961).

In the present study, neutron activation analysis was chosen for the following reasons (SOREMARK 1965; BOWEN 1966): (1) there is a high degree of sensitivity and specificity and (2) the risk of contamination and loss can be almost completely eliminated.

Material and Methods. The animals used in the present study were albino rats (Sprague-Dawley). All animals were normally lively and no signs of infection

deficiency, or any disease were observed. Only females were used, there were ten 3 week old and ten 24 week old rats. The young animals were housed together with their mothers in acrylic cages with steel covers until sacrifice. The mothers of the young rats and the 24 week old rats had free access to tap water and a pellet rat diet, Anticimex 210 (Anticimex, Norrviken, Stockholm) with a zinc content of about 73 ppm.

The rats were mildly anesthetized with ether and killed by decapitation. About 0.8 ml of blood was collected from each rat immediately after decapitation. The various tissues to be analysed were then rapidly excised in the following order: kidney, pancreas, spleen, liver (median lobe), heart, incisors, mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis. The tissues were not perfused. When possible, the whole organ was removed. The teeth examined were the four incisors, which were broken off at the gingival margin, thus, only the crowns were analysed. All visible pulp was removed. The mandibular bone sample consisted of the ramus and part of the corpus including both spongy and compact bone. The periosteum was scraped away. When removing the tibia diaphysis great care was taken to avoid the metaphyseal part. The diaphyseal part was dissected free, sectioned along its long axis, and freed of its bone marrow and periosteum. In this way, attempts were made to collect only the compact bone of the diaphysis. During the removal and preparation of the various specimens, no metal instruments were used. In order to avoid contamination only polyethylene or polyethylene covered instruments were in direct contact with the specimen. The specimens were collected in polyethylene tubes, and the wet weight was recorded immediately after removal of the tissue. The wet weights of the tissues varied between about 800 mg, e.g. blood and liver, and 15 mg, e.g. incisors and mandibular condyle. After weighing the specimens were dried in an electric oven for about 24 hours at 70 to 80°C in order to prevent volatilization during irradiation. Care was taken to avoid contamination of samples and standards before irradiation. The water used for dissolution of the standards to be sent for irradiation as well as that used for rinsing instruments and glassware was carefully purified according to the method described by SOREMARK & JOHANSSON (1963) and collected in polyethylene bottles.

A standard amount of zinc was inserted into a separate polyethylene tube and placed in the same aluminium can as the specimens. The aluminium can containing the standard and the specimens was irradiated by thermal neutrons for periods of 5 and 12 days in the R2 reactor of the Swedish Atomic Energy Company in Studsvik, Nyköping. The neutron fluence was approximately 1.4×10^{11} cm⁻² sec⁻¹.

When the period of irradiation was completed the samples were dissolved in hot H₂SO₄, followed by dropwise addition of 30% H₂O₂ per ml. Thereafter,

Table 2

Concentration of zinc in some hard and soft tissues from 3-week-old female rats — The values are based on wet weights and expressed in ppm \bar{x} = mean of 10 rats s = standard deviation

Tissue	\bar{x}	s	$100 s/\bar{x}$
Blood	6.3	1.1	17%
Kidney	18.6	2.9	16
Pancreas	30.0	5.7	19
Spleen	16.8	3.5	21%
Liver	33.0	18.3	35
Heart	14.6	4.3	29
Incisors	178.0	40.7	37
Mandibular condyle	152.0*	57.7	38
Mandibular bone	171.0	25.0	21
Tibia epiphysis	45.0	9.5	21
Tibia diaphysis	140.0	22.8	16

* One sample spoiled during preparation — mean value based on nine samples

Table 3

Concentration of zinc in some hard and soft tissues from 24-week-old female rats — The values are based on wet weights and expressed in ppm \bar{x} = mean of 10 rats s = standard deviation

Tissue	\bar{x}	s	$100 s/\bar{x}$
Blood	13.0	15.4	118
Kidney	18.4	4.2	23
Pancreas	27.6	19.8	88
Spleen	63.7	28.1	44
Liver	44.9	33.4	74
Heart	14.5	3.4	23
Incisors	111.0	33.0	30
Mandibular condyle	223.0	84.9	38
Mandibular bone	204.0	61.0	30
Tibia epiphysis	250.0	5.5	21
Tibia diaphysis	250.0	51.9	21

the solution was diluted with 0.7 N HCl p.a. The diluted solution flowed at a rate of 2 ml/min through an anion exchange column in chloride form Dowex 2 \times 10. The columns used were 10 cm high with a diameter of 1 cm. A faint suction was applied by means of a vacuum pump. After the dropping had ceased the column was washed with 50 ml of 0.7 N HCl p.a. in order to remove traces

Table 4

Some previous results on zinc concentration in various rat tissues compiled together with the results obtained in the present study — Mean values are given and the concentrations are expressed in ppm

Year	Author	Rat age in weeks	Method
1926	LUTZ		Fluorescence
1927	HILLER & BURKE		Fluorescence
1938	HOVE et coll		Colorimetry
1940	DAY & McCOLLUM	15	Colorimetry
1951	MAWSON & FISCHER		Colorimetry
1956	GILBERT & TAYLOR	6—12	Colorimetry
1958	MILLAR et coll	12—13	Colorimetry
1960	FORDES & VOIR		Colorimetry
1961	FORDES	9	Colorimetry
1961	TAYLOR	8—49	Colorimetry
1962	ALFVANDER & NUSBAUM	0—59	Roentgen ray fluorescence and emission spectrography
1966	HUXLEY & FLAVEL	14—15	Polarography
1966	MACAPINLAG et coll	10	Atomic absorption spectrophotometry
1967	KEINIGOLD et coll		Colorimetry
1967	PRASAD et coll	3	Atomic absorption spectrophotometry
1968	SWENLERTON & HURLEY		Atomic absorption spectrophotometry
Present study		3	Neutron activation analysis
		24	Neutron activation analysis

of ^{41}Ni . The standards were treated in exactly the same way as the biologic samples. The radiochemical separation method used was a modification of the one described by SAMSAHI et coll (1963).

After the radioactive zinc had been collected in the anion exchange column, the resin was transferred to stoppered polyethylene tubes for analysis in a 5" \times 3" well type NaI (Tl) scintillation detector connected to a transistorized 512 channel gamma spectrometer.

Quantitative data based on the wet weight of the organ samples were obtained by comparing the gamma intensity, the photoppeak area at 11156 MeV of ^{65}Zn

Table 4 (cont.)

Weight	Whole blood	Kidney	Pancreas	Spleen	Liver	Heart	Teeth	Bone
Wet	6.7	14.4		36.3	20.7			178 ±
Wet		15		31	20	14		92
Dry					76		97 (dent n and enamel)	237
Ash			23.3		30.3			233 (tibia)
Wet	3.88	23.4		24	29.7			133.9
Wet					43			285 (femur)
Wet								77—191
Ash								(femur)
Wet								323 (femur shaft)
Ash								420 (femur end)
Dry							200 (dent n) 130 (enamel)	388 (femur)
Wet		41			54			162 (femur)
Wet		19.4	17.5	16.7	93.9			168
Dry		91		105	101	73		424 (femur)
Ash								
Wet	6.3	18.6	30	16.8	53	14.6	128 (dent n and enamel)	140 (tibia diaphysis)
Wet	13	18.4	26	63.7	44.9	14.5	111 (dent n and enamel)	200 (tibia diaphysis)

or at 0.439 MeV of ^{65}Zn in the sample with that of the standard (MARINELLI *et coll* 1952).

The method used has been discussed by SAMSAHL & SOREMARK (1961), SOREMARK & BERGMAN (1962), SAMSAHL *et coll* (1963), BOWEN & GIBBONS (1963), OLFIN *et coll* (1966) and HALL *et coll* (1968). BRUNE (1963) reported a yield for ^{65}Zn of 98% with a standard deviation of 8% by the chemical group separation method developed by SAMSAHL *et coll* (1963). The loss of zinc due to the chemical procedures after the irradiation was also tested in the present work. To each of four hard and four soft tissue samples was added a known amount of ^{65}Zn . The samples then underwent the same chemical procedure as

the irradiated samples and were subjected to scintillation measurements. The yield was 97.3 % with a standard deviation of 3.5 %.

Various errors in the sampling method have been analysed elsewhere (BIRCHMAN 1970), and were found to be small. Therefore, it seems logical to assume that the large standard deviations obtained for some of the tissues in the present study — especially blood, pancreas and liver in the 24 week old rats — can be ascribed mostly to biological variations.

Results

The zinc concentrations obtained by means of neutron activation and gamma ray spectrometric analysis of some hard and soft tissues from 3- and 24 week old rats are presented in Tables 2 and 3.

Intra- and interindividual differences were tested by means of Student's *t* test. Except for tibia epiphysis in the 3 week animals, all the bone samples ($p < 0.001$) and the incisors ($p < 0.01$), had significantly higher zinc concentrations than the soft tissue samples within both age groups. For incisors, blood, kidney, pancreas, liver, and heart, there were no significant differences between the two age groups. The zinc concentration was significantly higher in adult rats in spleen ($p < 0.001$), mandibular bone ($p < 0.01$), tibia epiphysis ($p < 0.001$) and tibia diaphysis ($p < 0.001$). For mandibular condyle the zinc concentration was almost significantly higher in adult rats ($p \sim 0.05$).

Discussion

Comparisons of different studies concerning microelements such as zinc in biologic tissues are complicated for many reasons. The materials and methods vary and results are expressed in different ways, e.g. based on wet weight, dry weight, or ash weight. Furthermore, age is an important variable for the zinc concentration in skeletal samples.

For all the tissues analysed in the present study, except mandibular condyle and mandibular bone data on zinc concentrations in rats are available from earlier studies. In Table 4, some previously reported results for rat tissues are compiled together with the results obtained in the present study.

Where comparisons can be made, the results obtained in the present study agree comparatively well with those previously published. Spleen was the only soft tissue tested where a significant difference could be shown between the two age groups in the present study. At present no explanation can be given for this difference. It is also noteworthy that the zinc concentration in the spleen of the 24 week old rats differs considerably from previously published values (Table 4).

It was found in the present study that the skeletal samples of the adult rats

Table 5

Concentration of zinc in some hard tissues of female rats — The values in Tables 2 and 3 have been used and are here recalculated on ash weight and expressed in ppm — Numbers within parentheses indicate the mean ratios ash wt/wet weight each obtained from 6 rats \bar{x} = mean of 10 rats s = standard deviation

Tissue	3 week rats		24 week rats	
	\bar{x}	s	\bar{x}	s
T eeth	197 (65)	63	154 (72)	46
Mandibular condyle	700 (20)	242	572 (39)	218
Mandibular bone	404 (30)	83	371 (55)	111
Tibia epiphysis	566 (8)	188	730 (34)	154
Tibia diaphysis	300 (40)	46	391 (64)	81

had a significantly higher zinc concentration per gram wet weight than those of the young rats for mandibular condyle the difference was almost significant. The zinc concentrations found for skeletal tissues containing spongy bone (mandibular condyle, mandibular bone and tibia epiphysis) will also include zinc in the hemopoietic bone marrow. Zinc concentrations in bones from various ages have previously been analysed by TAYLOR (1961) and ALEXANDER & NUSBAUM (1962). TAYLOR (1961) reported that the mean zinc content of femur, humerus and pelvis in rats continued to rise from 56 days to 679 days from 77 up to 200 ppm wet weight. The zinc content of the ribs did not increase as much as that of the other bones. ALEXANDER & NUSBAUM (1962) contrary to TAYLOR (1961) were not able to show any elevation of zinc in rat bone with increasing age from newborn up to 414 days. These authors were also unable to show any correlation between age and zinc content in human ribs. They found it likely that the level of zinc in bone was directly related to the zinc to calcium ratio of the diet. The results of TAYLOR (1961) and those of the present study are based on wet weight while those of ALEXANDER & NUSBAUM (1962) are based on ash weight. This fact complicates direct comparisons between the studies. The bone of the adult animals give more ash per unit volume than that of young animals due to a higher degree of mineralization.

The following additional experiment was carried out in order to determine whether or not comparison is possible between results based on wet weight and

the irradiated samples and were subjected to scintillation measurements. The yield was 97.3 % with a standard deviation of 3.5 %.

Various errors in the sampling method have been analysed elsewhere (BERGMAN 1970), and were found to be small. Therefore, it seems logical to assume that the large standard deviations obtained for some of the tissues in the present study — especially blood, pancreas and liver in the 24 week old rats — can be ascribed mostly to biological variations.

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Tissue	3 week rats		74 week rats	
	\bar{x}	s	\bar{x}	s
Incisors	197 (65)	63	154 (72)	46
Mandibular condyle	750 (70)	242	572 (39)	218
Mandibular bone	404 (30)	83	371 (55)	111
Tibia epiphysis	566 (8)	188	730 (34)	154
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For all the tissues analysed in the present study, except mandibular condyle and mandibular bone data on zinc concentrations in rats are available from earlier studies. In Table 4 some previously reported results for rat tissues are compiled together with the results obtained in the present study.

Where comparisons can be made, the results obtained in the present study agree comparatively well with those previously published. Spleen was the only soft tissue tested where a significant difference could be shown between the two age groups in the present study. At present no explanation can be given for this difference. It is also noteworthy that the zinc concentration in the spleen of the 24-week old rats differs considerably from previously published values (Table 4).

It was found in the present study that the skeletal samples of the adult rats

Table 5

Concentration of zinc in some hard tissues of female rats — The values in Tables 2 and 3 have been used and are here recalculated on ash weight and expressed in ppm — Numbers within parentheses indicate the mean ratios ash wet weight/ash dry weight each obtained from 6 rats \bar{x} = mean of 10 rats s = standard deviation

Tissue	3 week rats		24 week rats	
	\bar{x}	s	\bar{x}	s
Inci ors	197	63	134	46
	(65)		(72)	
Mandibular condyle	713	247	572	218
	(20)		(39)	
Mandibular bone	404	83	371	111
	(30)		(33)	
Tibia epiphys	566	188	733	154
	(8)		(34)	
Tibial diaphysis	530	47	391	81
	(40)		(64)	

had a significantly higher zinc concentration per gram wet weight than those of the young rats for mandibular condyle the difference was almost significant. The zinc concentrations found for skeletal tissues containing spongy bone (mandibular condyle, mandibular bone and tibia epiphysis) will also include zinc in the hemopoietic bone marrow. Zinc concentrations in bones from various ages have previously been analysed by TAYLOR (1961) and ALEXANDER & NUSBAUM (1962). TAYLOR (1961) reported that the mean zinc content of femur, humerus and pelvis in rats continued to rise from 56 days to 679 days from 77 up to 200 ppm wet weight. The zinc content of the ribs did not increase as much as that of the other bones. ALEXANDER & NUSBAUM (1962) contrary to TAYLOR (1961) were not able to show any elevation of zinc in rat bone with increasing age from newborn up to 414 days. These authors were also unable to show any correlation between age and zinc content in human ribs. They found it likely that the level of zinc in bone was directly related to the zinc to calcium ratio of the diet. The results of TAYLOR (1961) and those of the present study are based on wet weight while those of ALEXANDER & NUSBAUM (1962) are based on ash weight. This fact complicates direct comparisons between the studies. The bone of the adult animals give more ash per unit volume than that of young animals due to a higher degree of mineralization.

The following additional experiment was carried out in order to determine whether or not comparison is possible between results based on wet weight and

those based on ash weight. Six 3 week old and six 24 week old rats were injected intraperitoneally with $0.2 \mu\text{Ci } ^{65}\text{Zn}$ per gram body weight and killed after 24 hours. The tibia diaphysis of the hind legs of each rat was dissected free and freed from bone marrow and periosteum, the wet weight was recorded, and the total concentration of ^{65}Zn in the bone samples determined. The ash weight was recorded after 16 hours at 600°C (ad modum ROBINSON & ELLIOT 1957, ALEXANDER & NUSBAUM 1962), and the total concentration of ^{65}Zn was determined in the ashed samples. By using metabolically incorporated ^{65}Zn in this way, it could be verified that no loss of zinc took place during the conditions of ashing. The ratio ash weight/wet weight was further recorded for the incisors, mandibular condyle, mandibular bone and tibia epiphysis. Using these figures, the zinc concentrations for the hard tissues (Tables 2 and 3) were recalculated on the basis of ash weights (Table 5). When the differences between 3 and 24 week old rats were tested statistically, only the mean ratios ash weight/wet weight were considered, as the standard deviations of these mean ratios were of negligible magnitude ($< 3\%$).

No significant differences could be found between the young and adult rats using the ash weights for the zinc concentrations in the incisors, mandibular condyle, mandibular bone or tibia diaphysis. For tibia epiphysis, the adult rats showed an almost significantly higher zinc concentration than the young rats ($p < 0.05$). The mean values for zinc concentration in ashed tibia diaphysis in the present study (350 and 391 ppm) agree well with those reported by MAWSON & FISCHER (1951) for ashed tibia (390 ppm = 233 ppm wet weight) and ALEXANDER & NUSBAUM (1962) for ashed femur shaft (223 ppm). Thus, it appears that zinc concentration in skeletal tissues and incisors of rats increases as mineralization proceeds, corresponding to the situation in the human enamel. Surface enamel in man has been reported to contain a higher concentration of mineral than the deeper layers (THEWIS 1940, SONI & BRUDEVOLD 1959, ANGMAR et coll 1963), and BRUDEVOLD et coll (1963) found zinc to be more concentrated in surface layers than in deeper layers of the enamel in man. The most striking difference obtained in the present study between young and adult rats was found for tibia epiphysis: 45 and 250 ppm zinc based on wet weights. This difference may reflect the late start in mineralization as indicated by the low ash weight at three weeks and the continuing replacement of existing epiphyseal cartilage with bone with increasing age.

No significant difference could be found in the present study between the incisors in the two age groups. This can probably be explained by the fact that the rat incisors are almost fully mineralized at 3 weeks of age as indicated by the ash weight/wet weight ratios, and thereafter are subject to continuous growth and abrasion.

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SUMMARY

The concentration of zinc (ppm/wet weight) was determined in the mandibular condyle and in some selected hard and soft tissues of 3- and 24-week-old rats by means of neutron activation and gamma-ray spectrometry. Except for tibia epiphysis in the 3-week-old animals, the skeletal samples and the incisors had significantly higher zinc concentration than the soft tissue samples in both age groups. Excepting the spleen, which had a significantly increased zinc concentration in adult rats, none of the soft tissues sampled showed significant differences in zinc concentrations between young and old rats. The skeletal samples of the adult rats had a significantly higher zinc concentration than those of the young rats. These increases were shown to result most probably from increasing mineralization with increasing age. No significant difference was found between the zinc concentrations of the incisors of the two age groups.

ZUSAMMENFASSUNG

Die Konzentration von Zink (ppm/Feuchtgewicht) wurde in der Mandibularcondyle und in verschiedenen ausgewählten harten und weichen Geweben von 3- und 24-Wochen alten Ratten mit Hilfe von Neutronenaktivierung und Gammastrahlen-Spektrometrie bestimmt. Mit Ausnahme der Epiphyse der Tibia von 3-Wochen alten Tieren war die Zinkkonzentration der Skelettproben und der Inzisoren in beiden Altersgruppen signifikant h her als in den Proben der weichen Gewebe. Mit Ausnahme der Milz, deren Zinkkonzentration bei erwachsenen Ratten signifikant angestiegen war, bestanden keine signifikanten Unterschiede der Zinkkonzentration der Proben weicher Gewebe zwischen jungen und alten Ratten. Die Skelettproben erwachsener Ratten hatten eine signifikant h here Zinkkonzentration als die der jungen Ratten. Dieser Anstieg ist am wahrscheinlichsten das Resultat der steigenden Mineralisation mit steigendem Alter. Es wurde keine signifikante Differenz zwischen der Zinkkonzentration der Inzisoren der beiden Altersgruppen gefunden.

R SUM 

L'auteur a d termin  par activation neutronique et par spectrom trie de rayons gamma la teneur en zinc (ppm/poids humide) dans le condyle mandibulaire et dans certains tissus durs et mous de rats ag s de 3 et de 24 semaines.   l'exception de l' piphyse tibiale des rats ag s de 3 semaines, les  chantillons osseux et les incisives avaient une teneur en zinc significativement plus  lev e que celle des  chantillons de tissu mou dans les deux groupes d' ge.   l'exception de la rate dont la teneur en zinc  tait significativement augment e chez les rats adultes, aucun des  chantillons de tissu mou ne pr sentait de diff rence significative de leur teneur en zinc entre les jeunes rats et les vieux rats. Les  chantillons osseux de rats adultes avaient une teneur en zinc significativement plus  lev e que celle de jeunes rats.

L'auteur a montré que cette élévation de la teneur en zinc résulte très probablement de l'augmentation de la minéralisation osseuse en fonction de l'âge. Il n'a pas constaté de différence significative de la teneur en zinc des incisives dans les deux groupes d'âge.

REFERENCES

- ALEXANDER V and NUSBAUM R F. Zinc in bone. *Nature (Lond)* 195 (1962), 903
- ANCIAR H, CARLSTROM D and GLAS J E. Studies on the ultrastructure of dental enamel. IV. The mineralization of normal human enamel. *J Ultrastruct Res* 11 (1963) 17
- BABICKY A and TAYLOR D M. Determination of zinc in human teeth using the activation analysis. *Cy Stomat* 66 (1966) 167
- BERNSTAM R A. A clinical and experimental investigation into the zinc content of plasma and blood corpuscles with special reference to infancy. *Acta paediat* (1952) Suppl No 87
- BERGMAN B. Comparative study of distribution of injected ^{65}Zn in the mandibular condyle and other tissues in rat as determined by gamma scintillation. To be published in *Acta radiol Ther Phys Biol* 9 (1970)
- BOQUIST L and LERNMARK Å. Effects on the endocrine pancreas in Chinese hamsters fed zinc deficient diets. *Acta path microbiol scand* 76 (1969) 215
- BOWEN H J M. The determination of copper and zinc in biological material by activation analysis. *Int J appl Radiat* 4 (1959) 211
- Trace elements in biochemistry. Academic Press, London and New York 1966
- and GIBBONS D. Radioactivation analysis. Clarendon Press, Oxford 1963
- BRUDEVOID F, STEADMAN L T, SPINELLI M A et coll. A study of zinc in human teeth. *Arch oral Biol* 10 (1963) 135
- BRUNE D. Report LF 4. Forskningsrådets Laboratorium (In Swedish). AB Atomenergi Studsvik, Sweden 1963
- BUTT E M, NUSBAUM R E, GILMOUR F C and DIDIO S L. Trace metal levels in human serum and blood. *Arch environm Hlth* 8 (1961) 52
- CRICKSHANK D B. The natural occurrence of zinc in teeth. *Brit dent J* 61 (1936) 530
- DAY H G and MCCOLLUM E V. Effects of acute dietary zinc deficiency in the rat. *Proc Soc exp Biol (N Y)* 45 (1940) 282
- FORDIS R M. Excretory patterns and bone deposition of zinc, calcium and magnesium in the rat as influenced by zinc deficiency. *FDTA and lactose J Nutr* 74 (1961) 194
- and YONE M. Zinc requirement and balance studies with the rat. *J Nutr* 70 (1960) 53
- GILBERT I G F and TAYLOR D M. The behaviour of zinc and radiozinc in the rat. *Biochim biophys Acta (Amst)* 21 (1956) 545
- GRIFFITH G C, BUTT F M and WALKER J. The inorganic element content of certain human tissues. *Ann intern Med* 41 (1954) 501
- HACKLEY B M, SMITH J C and HALSTEAD J A. A simplified method for plasma zinc determination by atomic absorption spectrophotometry. *Clin Chem* 14 (1968) 1
- HAHN K J, TUMA D J and SULLIVAN J L. Rapid and simple continuous radiochemical separation of copper, magnesium, zinc and manganese in biological materials. *Anal Chem* 40 (1968) 974
- HAUMONT S. Le zinc dans le tissu osseux. Editions Arscia, Bruxelles 1963
- et VINCENT J. Zn^{65} et calcification du squelette. *Experientia (Basel)* 17 (1961) 296
- HELLER V G and BURKE A D. Toxicity of zinc. *J biol Chem* 74 (1927) 85

- HOVE E ELVENJEM C A and HART E B Further studies on zinc deficiency in rats *Amer J Physiol* 174 (1938) 750
- HUXLEY H G and LEAVER A G The effect of different levels of dietary zinc and calcium upon the zinc concentration of the rat femur and incisor *Arch oral Biol* 11 (1966) 1337
- KENNAMON K E Some independent and combined effects of copper molybdenum and zinc on the placental transfer of zinc-65 in the rat *J Nutr* 81 (1963) 312
- LANDGREBE A R McSHARRY W O and CEROLA M The radiometric titration of trace amounts of zinc *Int J appl Radiat* 10 (1968) 23
- LEDDICOTTE G W MULLINS W T BATE L C et coll The use of neutron activation analysis in analytical chemistry Second U N Intern Conference on the Peaceful Uses of Atomic Energy New York June 1958
- LUNDBERG M SOREMARK R and THILANDER H Gamma ray spectrometric analysis of some elements in coronal dentine of unerupted (impacted) human teeth *Odont Revy* 16 (1965) 97
- LUTZ R E The normal occurrence of zinc in biologic materials a review of the literature and a study of the normal distribution of zinc in the rat cat and man *J industr Hyg* 8 (1926) 177
- MACAPINLAG M P PEARSON W N and DARBY W J Some characteristics of zinc deficiency in the albino rat *In Zinc metabolism* Chapter 8 Edited by A S Prasad Charles C Thomas Springfield Illinois 1966
- MALMSTROM B G Determination of zinc in biological material *Meth biochem Anal* 3 (1956) 327
- MARNELLI L D MILLER C E MAY H A and ROSE J E Low level gamma ray scintillation spectrometry experimental requirements and biomedical applications *In Advances in biological and medical physics* Volume 8 Edited by C A Tobias and J H Lawrence Academic Press New York 1962
- MAWSON C A and FISCHER M I Zinc content of the genital organs of the rat *Nature (Lond)* 167 (1951) 859
- MEINKE W W Trace-element sensitivity Comparison of activation analysis with other methods *Science* 121 (1955) 177
- MILLAR M J FISCHER M I ELCOATE P N and MAWSON C A The effects of dietary zinc deficiency on the reproductive system of male rats *Canad J Biochem* 36 (1958) 557
- MONACELLI R TANAKA H and YOR J H Spectrochemical determination of magnesium chromium nickel copper and zinc in human plasma *Clin chim Acta* 1 (1956) 577
- NIXON G S LIVINGSTON H D and SMITH H Estimation of zinc in human enamel by activation analysis *Arch oral Biol* 12 (1967) 411
- OLEHY D A SCHMIDT R A and BETHARD W F Neutron activation analysis of magnesium calcium strontium barium manganese cobalt copper zinc sodium and potassium in human erythrocytes and plasma *J nucl Med* 7 (1966) 917
- PARR R M and TAYLOR H M The concentrations of cobalt copper iron and zinc in some normal human tissues as determined by neutron activation analysis *Biochem J* 91 (1964) 424
- PFELSTICKER K Spurenelemente in Organen und Urin bei Krebs *Z klin Chem* 3 (1965) 145
- PHIL M and GLISTAFSON G T Heavy metals in rat mast cell granules *Lab Invest* 17 (1967) 588
- PRASAD A S OVERLEAS D and HALSTED J A Determination of zinc in biological fluids by atomic absorption spectrophotometry in normal and cirrhotic subjects *J Lab clin Med* 65 (1965) 508

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REFERENCES

- ALEXANDER V and NUSBAUM R F Zinc in bone *Nature* (Lond) 195 (1962) 903
- ANCMAR B CARLSTROM D and CLAS J F Studies on the ultrastructure of dental enamel
IV The mineralization of normal human enamel *J Ultrastruct Res* 8 (1963) 12
- BABICKY A and TAYLOR D M Determination of zinc in human teeth using the activation analysis *Cs Stomat* 66 (1966), 167
- BERGFSTAM R A A clinical and experimental investigation into the zinc content of plasma and blood corpuscles with special reference to infancy *Acta paediat* (1952) Suppl No 87
- BERGMAN B Comparative study of distribution of injected ^{65}Zn in the mandibular condyle and other tissues in rats determined by gamma scintillation To be published in *Acta radiol Ther Phys Biol* 9 (1970)
- BOQUIST L and IFRMARK A Effects on the endocrine pancreas in Chinese hamsters fed zinc deficient diets *Acta path microbiol scand* 76 (1969) 215
- BOWEN H J M The determination of copper and zinc in biological material by activation analysis *Int J appl Radiat* 4 (1959) 211
- Trace elements in biochemistry Academic Press London and New York 1966
- and GIBBONS D Radioactivation analysis Clarendon Press Oxford 1963
- BRUDINOLD I STRADMAN I J SPINELLI M A et coll A study of zinc in human teeth *Arch oral Biol* 8 (1963) 139
- BRUN D Report Li 1 Forskningsrådets Laboratorium (In Swedish) AB Atomenerg Studsvik Sweden 1963
- BUTT F M NUSBAUM R E GILMOUR T C and DIDIO S L Trace metal levels in human serum and blood *Arch environm Hlth* 8 (1961) 52
- CRUCIFIANAK D B The natural occurrence of zinc in teeth *Brit dent J* 61 (1936) 530
- DAY H G and MCCOLLUM F V Effects of acute dietary zinc deficiency in the rat *Proc Soc exp Biol (N Y)* 45 (1910) 282
- LORDS R M Secretory patterns and bone deposition of zinc calcium and magnesium in the rat as influenced by zinc deficiency *PDTA and lactose J Nutr* 74 (1961) 194
- and YOSHIMIZU M Zinc requirement and balance studies with the rat *J Nutr* 70 (1960) 53
- GILBERT I G F and TAYLOR D M The behaviour of zinc and radiozinc in the rat *Biochim biophys Acta (Amst)* 21 (1956) 303
- GRIFFITH G C BUTT F M and WALSH J The inorganic element content of certain human tissues *Ann intern Med* 41 (1954) 501
- HACHIMY B M SMITH J C and HALLSTAD J A A simplified method for plasma zinc determination by atomic absorption spectrophotometry *Clin Chem* 14 (1968) 1
- HALLIN K J TOMA D J and SULLIVAN J L Rapid and simple continuous radiochemical separation of copper magnesium zinc and manganese in biological materials *Anal Chem* 40 (1968) 974
- HAUMONT S Le zinc dans le tissu osseux Editions Arscia Bruxelles 1963
- et VINCENT J Zn^{65} et calcification du squelette *Experientia* (Basel) 17 (1961) 296
- HALL V G and BURKE A D Toxicity of zinc *J biol Chem* 71 (1927) 85

RADIOPATHOLOGY OF AMERICIUM 241

1 Distribution of americium in adult mice

by

LARS HAMMARSTROM and AGNAR NILSSON

The increasing production and use of transuranic elements have enhanced the interest in their relative toxicities metabolic behaviour and carcinogenic properties. At present the maximum permissible levels for transuranic elements are based on the assumption that their biologic risks are similar to those of plutonium however this does not always seem to be true. A comparison of the uptake of ^{241}Am and ^{239}Pu in the skeleton of rats indicated that the initial uptake of ^{239}Pu in the skeleton was about 1.4 times greater than that of ^{241}Am while the rate of loss from the skeleton was almost the same for these nuclides. The rate of excretion from the body was higher for ^{241}Am than for ^{239}Pu and the retention of the latter in the liver was of a longer duration than that of ^{241}Am (TAYLOR et coll 1961).

Differences also exist in terms of biologic effects. BENSTED et coll (1965) have proved that 77% (17/22) of rats given 3.0 μCi ^{239}Pu /kg body weight developed bone tumours. In addition one case of renal carcinoma and one of myelogenous leukemia were observed. After the injection of 2.5 μCi ^{241}Am /kg body weight

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- WOLF P and HORWITZ J P Studies on zinc deficiency Changes in trace elements and enzyme activities in tissues of zinc deficient rats *J clin Invest* 46 (1967), 549
- REINHOLD J G, KFOURA G A and THOMAS T A Zinc copper and iron concentrations in hair and other tissues Effects of low zinc and low protein intakes in rats *J Nutr* 92 (1967), 173
- ROBINSON R A and ELLIOT S R The water content of bone I The mass of water inorganic crystals organic matrix and CO_2 space components in a unit volume of dog bone *J Bone Jt Surg* 39 A (1957) 167
- SAMSAHL K A chemical eight group separation method for routine use in gamma spectrometric analysis 1 and 2 Reports AF 54 and 56 1961 AB Atomenergi Studsvik Sweden
- and SOREMARK R Comparative and absolute measurements of 11 inorganic constituents of 38 human tooth samples with gamma ray spectrometry Proc Intern Conference on Modern Trends in Activation Analysis December 1961 College Station Texas
- BRUNE D and WESTER P O Simultaneous determination of 30 trace elements in cancerous and noncancerous human tissue samples with gamma ray spectrometry Report AE 174 1963 AB Atomenergi Studsvik Sweden
- SCOLLAR I I A quantitative study by means of spectrographic analysis of zinc in nutrition *J Nutr* 17 (1939) 103
- SONI N N and BRUDFOLD F Microradiographic and polarized light studies of initial carious lesions *J dent Res* 38 (1959) 1187
- SOREMARK R Microelement analysis by neutron activation In Progress in atomic medicine Edited by J H Lawrence Grune & Stratton New York 1965
- and BERGMAN B Gamma ray spectrometric analysis of some micro elements in human mandibular compact bone *Acta isotop* 2 (1962) 5
- and JOHANSSON B Report LMF 1 Forskningsrådets laboratorium (In Swedish) AB Atomenergi, Studsvik Sweden 1963
- STRAIN W H STEADMAN L T LANKAU JR C A et coll Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man *J Lab clin Med* 68 (1966) 244
- SWERNERTON H and HURLEY L B Severe zinc deficiency in male and female rats *J Nutr* 95 (1968), 11
- TAYLOR D M Retention of zinc 65 in the bones of rats *Nature (Lond)* 189 (1961) 932
- THEWLIS J The structure of teeth as shown by X ray examination *Spec Rep Ser med Res Coun (Lond)* (1910) No 238
- VALLEE B L and GIBSON J G The zinc content of normal human whole blood plasma leukocytes and erythrocytes *J biol Chem* 176 (1948) 145
- WOLFF H Untersuchungen zur Pathophysiologie des Zinkstoffwechsels *Klin Wschr* 34 (1956) 409
- ZEITZ L and LEE R Zinc analysis in biological specimens by X ray fluorescence *Analyt Biochem* 14 (1966) 191

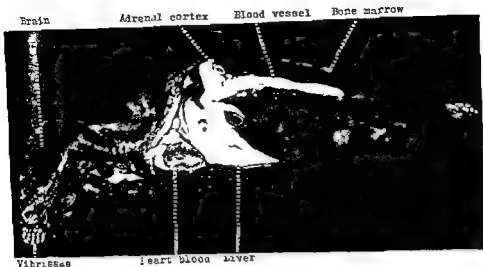


Fig 2 Autoradiogram Distribution of ^{241}Am in a male mouse 30 minutes after intravenous injection. High concentration in the liver and adrenal cortex, the concentration in the blood is lower than in the liver, no demonstrable radioactivity in the bone but a moderate concentration in the marrow.

freezing in hexane cooled with solid CO_2 (-70°C). One male mouse was killed at each of the time intervals of 5 minutes, 30 minutes, 24 hours, 4 days and 15 days after injection. Two mice were killed 30 days after injection and two after 60 days. The female mice that were injected on the 15th day of gestation were killed 24 hours and 4 days after injection and the two that were injected on the 18th day of pregnancy were killed 4 hours and 24 hours after injection.

The animals immediately before freezing were placed in an aqueous solution of carboxy methylcellulose applied on a large microtome stage. The specimens after freezing in hexane CO_2 were thus ready for sectioning which was performed in a freezebox (-15°C). To obtain sections through the whole animals, adhesive tape (No. 810 Minnesota Mining and Manufacturing Co.) was attached to the exposed surface of the frozen specimen before cutting; the sections then came off adhering to the tape. Sections $20\ \mu$ thick were taken and freeze dried in the box for 2 days after which they were brought to room temperature in an air tight box. The sections were then pressed against a roentgen film (Structurix D7, Gevaert) and exposed for four weeks. After the sections had been removed, the films were developed in G 230 and fixed in D 303 (Gevaert); the sections were stained with hematoxylin and eosin. The autoradiographic procedure has been described in detail by ULLBERG (1954, 1958).

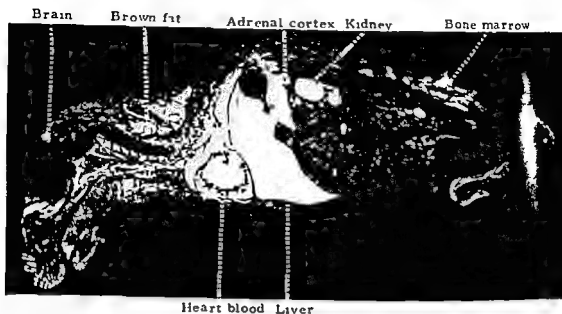


Fig 1 Autoradiogram Distribution of ^{241}Am in a male mouse 5 minutes after intravenous injection. High concentration (light areas) in the blood and liver as well as in the adrenal cortex; no demonstrable radioactivity in the bone but the marrow displays a moderate concentration.

only four out of nineteen rats (21%) developed osteosarcomas. Adrenal tumours were detected in two animals and one animal developed leukaemia of an undetermined type.

As part of an investigation of the pathologic effects of ^{241}Am in mice the present study of the distribution of this nuclide was performed in order to obtain more information concerning the organs at risk.

Material and Methods

Labelled compound Americium 241 with a concentration of 3.8 $\mu\text{Ci/ml}$ was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England. The radioactive compound was administered as a nitrate.

Animals Nine adult male mice and four pregnant female mice of the CBA strain were used. Two of the female mice were injected on the 15th day of gestation and the other two female mice on the 18th day of gestation. The average weight of the male mice was 20 g and of the pregnant mice 30 to 35 g.

Autoradiographic procedure Each animal was given 0.26 ml of the solution of ^{241}Am , corresponding to 1 μCi intravenously in a tail vein. After predetermined survival periods the animals were anesthetized with ether and killed by

Bone There was a latency in the uptake of americium in the bone. After 4 hours no deposition at the endosteal and periosteal surfaces of bone was observable and after 24 hours there was a high concentration at these sites. The concentration at the endosteal surfaces was usually higher than at the periosteal surfaces. The concentration as well as the localization seemed to be unchanged during the remaining investigation period. The bone marrow had a moderate concentration of ^{241}Am at all the intervals studied.

Cartilage Americium was taken up in the tracheal and auricular cartilage. As in bone there was a latency of some hours in the uptake which was limited to the surface. The intervertebral discs had no detectable amounts of the injected ^{241}Am .

Teeth A marked accumulation was observed in the dental pulp and in the periodontal membrane (cf Fig 5). There seemed to be some radioactivity at the surface of the developing enamel of the incisors.

Gastro intestinal tract Some radioactivity was seen in the contents of the stomach close to the secretory mucosa in all the animals studied and a small amount of radioactivity was also present in the intestinal lumen after long survival periods. No radioactivity appeared in the gastric or intestinal mucosa however. Five minutes after injection the concentration of ^{241}Am in the liver was the same as in the circulating blood. It then seemed to increase slightly and at all the intervals studied was the highest in the body. The distribution was fairly even shortly after injection but with time a redistribution towards a higher concentration around the central veins occurred. Americium was never observed in the gall bladder (cf Fig 6). The salivary glands and the pancreas never had any observable amounts of ^{241}Am .

Respiratory tract The concentration in the lungs followed that of the circulating blood. A high concentration was recorded in the bronchial cartilage however.

Urinary tract The whole kidney exhibited a moderate concentration of radioactivity and in addition a higher accumulation was observed in small spots in the renal cortex. No accumulation was present in the renal pelvis but radioactivity over the mucosa of the ureter and urethra persisted up to 60 days after the injection.

Endocrine organs The adrenal cortex had a fairly high concentration which persisted 60 days after the injection. At 5 minutes it had become evenly distributed. Twenty four hours and more after the injection the concentration in an outer zone presumably the zona glomerulosa exceeded that of the other zones of the cortex. This higher concentration in the outer zone was not present in the female mice. No radioactivity was observed in the adrenal medulla. A low concentration was present in the primary thyroid and pancreatic islets. Some radio-

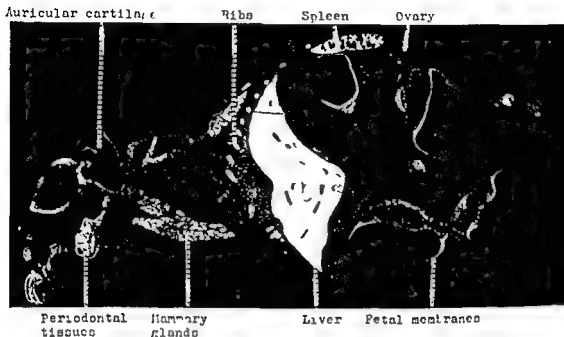


Fig 3 Autoradiogram Distribution of ^{241}Am in a pregnant mouse (19th day of gestation) 24 hours after intravenous injection Uptake in the liver endosteal and periosteal parts of the bone periodontal tissues auricular cartilage fetal membranes mammary glands and red pulp of the spleen

Results

Shortly after the intravenous injection of americium the highest concentration appeared in the blood and the liver. Some other richly vascularized tissues, such as the bone marrow, spleen, kidney, adrenal cortex, lungs, brown fat and nasal mucosa had a moderate concentration. No radioactivity was noted in the mineralized tissues, shortly after injection (Fig 1).

The concentration in the blood decreased during the first few hours and after 4 hours radioactivity was no longer discernible. The liver and the skeletal tissues seemed to be the major sites of deposition. A high and persistent uptake of radioactivity was also seen in the adrenal cortex, a few ovarian follicles, the marginal sinuses of the spleen, and the dental pulp. The distribution pattern remained fairly unchanged during the whole investigation period.

The distribution in the different tissues will be described more in detail below.

The excretion of the injected americium appeared to occur slowly, only a slight decrease of radioactivity in the organism was autoradiographically observable 30 to 60 days after injection.

Blood The concentration of americium was high shortly after injection but decreased rapidly to a level of 10% of the initial concentration within 24 hours.

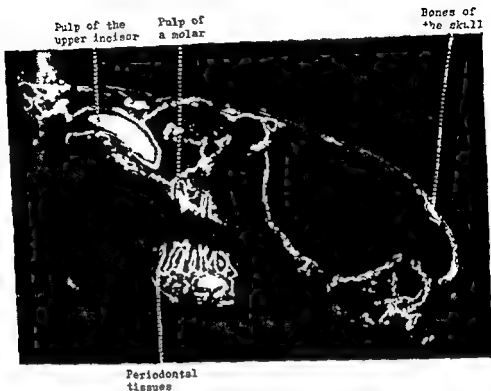


Fig 5 Autoradiogram Distribution in the head of a mouse 24 hours after intra-ear injection of ^{241}Am . High concentration in the bone, periodontal tissues and dental pulp

activity in these glands seemed to persist when no radioactivity was demonstrable in the circulating blood

Gonads The uptake in the testes was low but appeared to increase moderately with time. The radioactivity was mainly localized in the interstitial tissue. The ovaries of the pregnant mice had a high concentration in some follicles and a moderate concentration in the interstitium while the corpora lutea displayed a lower concentration (Fig 4)

The central nervous system presented no evidence of radioactivity

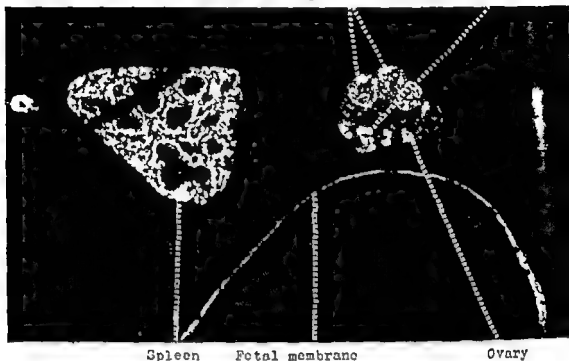
Lymphatic tissues The red pulp of the spleen had a fairly high concentration which seemed to remain unchanged during the whole period of investigation. The marginal sinuses had a higher concentration than the red pulp (Fig 4). No radioactivity was present in the white pulp of the spleen or in the lymph glands

Muscles The muscles had no detectable concentration

Skin and subcutaneous tissue The vibrissa follicles had an uptake of americium that gradually disappeared during the first day after the injection (Fig 2). No

Corpora lutea

Follicle walls



Spleen

Fetal membrane

Ovary



Gastric mucosa

Pancreas

Head of a fetus

Fig 4 Detail of autoradiogram (upper image) of a pregnant mouse (15th day of gestation) 4 hours after intravenous injection of ^{113}m and the corresponding stained section (lower image) depicting the distribution of ^{113}m in the ovary, spleen and adjacent placenta: high uptake in some follicle walls of the ovary

however a moderate concentration was noted in the fetal membranes (Figs 3 and 4)

Mammary glands had a moderate concentration at all the intervals studied

Fetuses Only little radioactivity was seen in the fetuses and only a faint representation of the skeleton was obtained in the animals injected at the 18th day of gestation

Discussion

The distribution of americium in the present investigation was predominantly characterized by an accumulation of the isotope in the bone and liver tissues. This is in close agreement with results obtained earlier with the impulse counting technique (SCOTT et coll 1945) and seems generally to be a common feature for all the actinide elements (TAYLOR 1964). The preferential accumulation of americium at the endosteal surfaces of bone appears to be compatible with previous findings that its concentration is higher on resting and resorbing surfaces of bone than on those where bone formation is in progress (TAYLOR et coll 1966, HERRING et coll 1962). The mechanism of binding of americium to bone tissue is not known although some evidence that it is bound to bone glycoproteins (HERRING et coll 1962, CHIPPERFIELD & TAYLOR 1968) exists. The finding in the present investigation that there was a latency in the uptake of the isotope in bone after an intravenous injection may indicate that americium is incorporated into a larger molecule that has an affinity for skeletal tissues.

Certain new sites of marked accumulation were also detected in the present investigation i.e. the adrenal cortex, the ovary and the dental pulp. The accumulation in the adrenal cortex may be placed in relation to the americium induced adrenal tumours in rats observed by BEASLEY et coll (1965). However ^{241}Am is not taken up in the adrenal cortex of young rats (HAMMARSTRÖM & NILSSON to be published). This may indicate that the binding mechanism is in some way associated with steroid hormone production after sexual maturation. Of other actinide elements studied both plutonium 239 (ULLBERG et coll 1962) and uranium 233 (WALINDER et coll 1965) have been reported to be accumulated in the adrenal cortex. The relative concentration in the adrenal cortex seems however to be considerably less for these two radioelements. Like americium also these two actinides were accumulated and retained in the ovary and the interstitial cells of the testes. Local irradiation of the reproductive cells may have grave genetic consequence. Our preliminary results concerning the pathologic effect of ^{241}Am have revealed serious atrophy of the testes.

The accumulation in the dental pulp noted in this investigation does not seem to have been observed for other actinide elements. The concentration

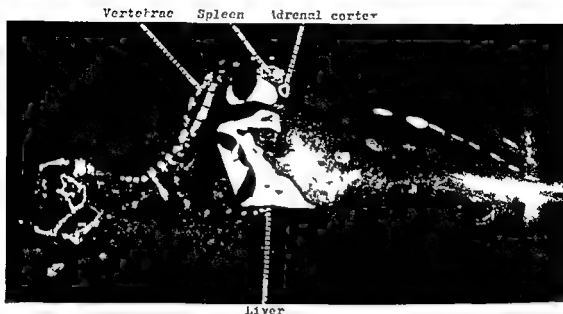


Fig 6 Autoradiogram Distribution of ^{211}Am in a mouse 1 day after intravenous injection. Attention may be drawn to the outer zone of the adrenal cortex and the marginal sinuses of the spleen.

uptake was noted in other hair follicles or in other parts of the skin or subcutaneous tissue.

Brown fat. A moderate concentration was seen in the brown fat. The disappearance from this tissue was slower than from the blood.

Placenta. No radioactivity was present in the placenta at the intervals studied.

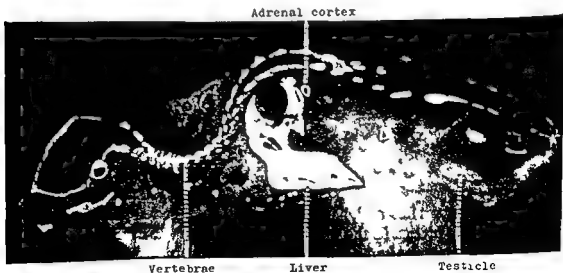


Fig 7 Autoradiogram Distribution of ^{211}Am in a mouse 60 days after intravenous injection. Attention may be drawn to the adrenal cortex, liver and bone.

ROENTGEN RAY EFFECTS ON THE OVARIES OF FOETAL MICE

by

B. HENRICSON and A. NILSSON

The effect of external irradiation on the gonads of post natal female mice has been the subject of numerous investigations (OAKBERG 1958 1960 1962 1966 1968 PETERS 1961 PETERS & BORUM 1961 PETERS & LEVY 1964 RUSSELL & RUSSELL 1956 PARSONS 1962 and others). When exposing the foetal ovary of rats to roentgen rays (50 to 100 R) BEAUMONT (1962 1966) found an increasing sensitivity of the germ cells between the 8th and 15th days of foetal life. In mice MINTZ (1959) revealed a very sensitive period on the 11th and 12th days of foetal life. The dose given was 100 to 300 R.

Concerning internal emitters NILSSON & HENRICSON (1969) have reported on the effect of 20 μCi ^{90}Sr per mouse given intravenously on the 11th or 16th day of intrauterine life. The effect was clearly higher on the 16th day.

The object of the present investigation was to study the effect of two doses of roentgen treatment (20 and 80 R) on the 11th day of uterine life and to compare it to the ^{90}Sr treatment.

appeared to be about the highest in the body and radiation injuries might well be expected. It would appear that a primary tumour of the dental pulp has never been reported and special attention will be paid to this tissue in a current long time study of the radiopathology of americium 241.

SUMMARY

The distribution and retention in mice of ^{241}Am after a single intravenous injection were investigated by autoradiography. The technique is described and the findings are discussed in detail.

ZUSAMMENFASSUNG

Die Verteilung und Aufspeicherung des ^{241}Am wurde mittels Autoradiographie nach einer einzelnen intravenösen Injektion studiert. Die Technik der Methode und deren Resultate werden im einzelnen beschrieben.

RÉSUMÉ

La répartition et la fixation du ^{241}Am chez les souris après une injection intra veineuse unique ont été étudiées par autoradiographie. Les auteurs décrivent la technique et analysent en détail les résultats.

REFERENCES

- BENSTED P. M., TAYLOR D. M. and SOWBY F. D. The carcinogenic effects of americium 241 and plutonium 239 in the rat. *Brit J Radiol* 38 (1965) 920.
- CHIFFERFIELD A. R. and TAYLOR D. M. Binding of plutonium and americium to bone glycoproteins. *Nature* 219 (1968) 609.
- HAMMARSTROM L. E. and NILSSON A. Radiopathology of americium 241. II Uptake in the developing teeth of rats. To be published in *Acta radiol Ther Phys Biol*.
- HERRING G. M., VAUGHAN J. and WILLIAMSON M. Preliminary report on the site of localization and possible binding agent for yttrium, americium and plutonium in cortical bone. *Hlth Phys* 8 (1962) 717.
- SCOTT K. G., COPE D. H., AYELROD D. J. and HAMILTON J. G. The metabolism of americium in the rat. *J Biol Chem* 175 (1945) 691.
- TAYLOR D. M. The metabolism of the transuranic elements. In: *Symp on The metabolism of biologically important radionuclides*. *Brit J Radiol* 37 (1964) 95.
- SOWBY F. A. and KEMMER N. F. The metabolism of americium and plutonium in the rat. *Phys in Med Biol* 6 (1961) 73.
- UHLBERG S. Studies on the distribution and fate of S^{35} labelled benzylpenicillin in the body. *Acta radiol* (1954) Suppl No 118.
- Autoradiographic studies on the distribution of labelled drugs in the body. Second UN Int Conf Peaceful Uses of Atomic Energy 24 (1958) 248.
- NELSON A., KRISTOFFERSSON H. and ENGSTROM A. Distribution of plutonium in mice. An autoradiographic study. *Acta radiol* 58 (1962) 459.
- WALINDER G., HAMMARSTROM L. and BILLAUDELL U. Incorporation of uranium. I Distribution of intravenously and intraperitoneally injected uranium. *Brit J Industr Med* 24 (1967) 305.

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- HAMMARSTROM L. E. and NILSSON A. Radiopathology of americium 241. II. Uptake in the developing teeth of rats. To be published in *Acta radiol. Ther. Phys. Biol.*
- HERRING G. M., VAUGHAN J. and WILLIAMSON M. Preliminary report on the site of localization and possible binding agent for yttrium, americium and plutonium in cortical bone. *High Phys.* 11 (1962) 717.
- SCOTT K. G., COPP D. H., AXELROD D. J. and HAMILTON J. G. The metabolism of americium in the rat. *J. Biol. Chem.* 175 (1945) 691.
- TAYLOR D. M. The metabolism of the transuranic elements. In *Symp. on The metabolism of biologically important radionuclides*. *Brit. J. Radiol.* 37 (1964) 95.
- , SOWBY B. A. and KEMPER N. F. The metabolism of americium and plutonium in the rat. *Phys. in Med. Biol.* 11 (1961) 73.
- ULLBERG S. Studies on the distribution and fate of S^{35} labelled benzylpenicillin in the body. *Acta radiol.* (1954) Suppl. No. 118.
- , Autoradiographic studies on the distribution of labelled drugs in the body. Second U.N. Int. Conf. Peaceful Uses of Atomic Energy 24 (1958) 248.
- , NELSON A., KRISTOFFERSSON H. and ENGSTROM A. Distribution of plutonium in mice. An autoradiographic study. *Acta radiol.* 58 (1962) 459.
- WALINDER G., HAMMARSTROM L. and BILLAUDELLE U. Incorporation of uranium. I. Distribution of intravenously and intraperitoneally injected uranium. *Brit. J. Industr. Med.* 24 (1967) 303.

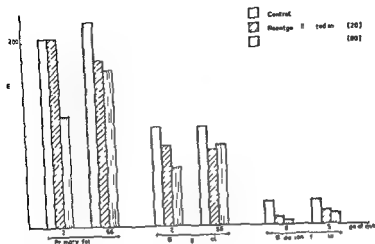


Fig 3 Average number of primary growing and Graafian follicles

The ovaries of the animals were immediately removed after sacrifice and fixed in Stieve's fluid. The right ovary was serially sectioned at $5 \mu\text{M}$. Every tenth section was investigated after staining with Ehrlich's haematoxylin-eosin.

The following types of oocytes and follicles were identified and counted:

Oocytes group I Oocytes without follicular cells

Oocytes group II Oocytes with 1 to 4 follicular cells

Oocytes group III Oocytes with 5 follicular cells to an almost complete single layer of follicular epithelium

Primary follicles Oocytes surrounded by one complete layer of follicular cells

Growing follicles Two or more layers of follicular cells without complete formation of an antrum

Graafian follicles Complete antrum formation in the follicular epithelium

Atretic follicles Pyknotic and lytic cells in the follicular layers usually containing a shrunken oocyte with disintegration of zona pellucida

Corpus atreticum The visible end result of atresia: a highly degenerated oocyte surrounded by a plasma membrane and peripheral to it some theca cells and connective tissue

Corpus luteum Growing follicles with two layers of follicular cells; the intercellular tissue in the stratum granulosa is loose and may represent the initiation of formation of large pool of follicular liquid characteristic of Graafian follicles

The total number of cells per ovary in each group of mice was calculated by using the formula of ABERCROMBIE (1946)

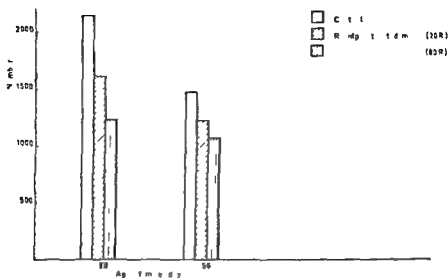


Fig. 1 Total number of oocytes and follicles. Average of five mice irradiated on the 11th day of foetal life and killed 28 or 56 days after birth.

Material and Methods Inbred CBA mice were irradiated on the 11th day of foetal life. The doses were 20 or 80 R. The roentgen equipment was a Muller MG 300 apparatus operated at 260 kV, 10 mA, focal distance 45 cm, inherent filtration 4 mm Al, additional filter 0.5 mm Cu, giving a dose rate of 75 R/min. Groups of five mice, including also unirradiated controls, were killed at 28 or 56 days after delivery.

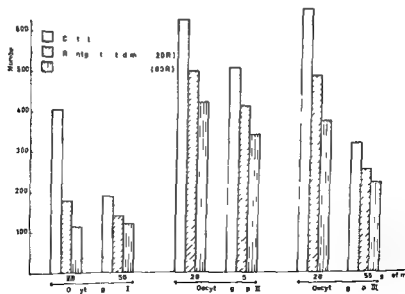


Fig. 2 Average number of oocytes of different types.

following 100 R. In our experiments with mice the reduction of oocytes was about 25 % after 20 R and 45 % after 80 R when estimating the numbers 28 days after birth. There is consequently some indication of higher sensitivity of primordial germ cells in the rat than in the mouse. However in BEALMONT's series the dose rate was about three times higher than in our experiment.

When evaluating the number of oocytes and follicles at the age of 56 days however we found a smaller decrease in cells than at the age of 28 days. The reduction at 56 days amounted to 19 % after 20 R and 28 % after 80 R. There seems consequently to have been some repair between 28 and 56 days of age.

In an earlier experiment (NILSSON & HENRICSON 1969) female mice were treated with a ^{90}Sr dose of 20 μCi per mouse on the 11th day of pregnancy. The killing effect on primordial germ cells was far greater than with even the highest roentgen dose (80 R) used in the present experiment. Following ^{90}Sr the reduction of oocytes and follicles at the age of 28 days was about 64 % and 56 days 58 %.

SUMMARY

Female mice were irradiated on the eleventh day of pregnancy with roentgen radiation of 20 R or 80 R. The female offspring were killed at the age of 28 or 56 days and oocytes and follicles were counted. There was a clear dose related reduction of the oocytes amounting to between 25 and 45 % in comparison with the controls. The fact that the reduction was less at the age of 56 days than at 28 days suggests a repairing process.

ZUSAMMENFASSUNG

Weibliche Mäuse wurden mit Dosen von 20 R oder 80 R am elften Tage der Schwangerschaft bestrahlt. Die weiblichen Nachkommen wurden im Alter von 28 oder 56 Tagen getötet und die Anzahl von Oocyten und Follikeln wurde gezählt. Eine deutliche dosis abhängige Reduktion der Oocyten zwischen 25 % und 45 % im Vergleich zu den Kontrollen wurde festgestellt. Die Reduktion war verhältnismässig gering bei den 56-Tage alten Mäusen als bei den 28 Tage alten, was auf einen Wiederholungsprozess deutet.

RÉSUMÉ

Des souris femelles ont reçu au onzième jour de leur gravidité des doses de rayons de Roentgen de 20 R ou de 80 R. Les souriceaux femelles ont été tués à l'âge de 28 ou de 56 jours et on a compté leurs oocytes et leurs follicules. La réduction du nombre des oocytes est nettement en relation avec la dose et se situe entre 25 et 45 % par rapport aux sujets témoins. Le fait que la réduction du nombre des oocytes est moins importante à l'âge de 56 jours qu'à 28 jours fait penser qu'il y a un processus de réparation.

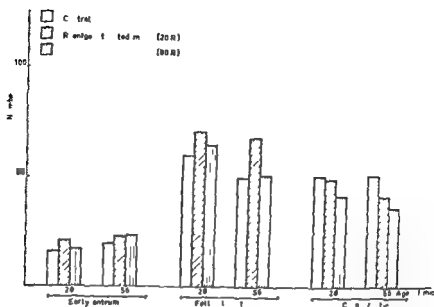


Fig. 4. Average number of early antrum (cf. text) and degenerated follicles.

Results

The total number of cells and follicles per ovary among the irradiated mice was greatly diminished (Fig. 1) compared with that of the control group. It was also obvious when comparing irradiated with non irradiated mice that the relative decrease in cells was much more marked after 28 than after 56 days. On the other hand, the reduction in the number of cells between day 28 and day 56 was greater in the control material than in the 20 R and 80 R groups (Fig. 1).

The radiation induced reduction comprised all types of oocytes and follicles (Figs 2 and 3) but in opposite tendency was observed among the early antrum follicles and degenerating follicles (Fig. 4).

The dose effect was revealed for some types of cells and follicles but not in all. The difference between the dose effects seemed to be greater in the 28 day than in the 56 day old mice.

Discussion

MINTZ (1959) irradiated female mice with 100 and 300 R at the same foetal age as we have used. As the effect was studied 2 to 3 days after exposure the evaluation cannot be compared to our investigation. In rats, however, a comparable experiment has been made by BEAUMONT (1962). She irradiated female rats at the foetal age of 10 and 12 days and counted the oocytes 25 days after birth. There was a reduction of about 40% following 50 R and 50 to 60%

EARLY EFFECTS OF LOCALIZED SINGLE DOSES OF IONIZING RADIATION ON HUMAN BONE MARROW

by

S STEFANI and A MONTE

Only sporadic observations are available on the effect of localized irradiation on the human bone marrow. All have been carried out during and after repetitive therapeutic irradiations (GOSWITZ ANDREWS & KNISELEY 1963 HUTAFF & BELDING 1955 LEHAR et coll 1966 STEWART & DISCHE 1956 SIKES et coll 1960). To our knowledge there are no reports on the effect of single localized doses of ionizing irradiation on the differential cell count of human bone marrow in the immediate hours and days following exposure. In order to supply this information the quantitative and qualitative changes in irradiated bone marrow of cancer patients were studied as a function of dose with constant time (two days after irradiation) and then as a function of time (4 hours 1 2 3 and 6 days) with constant dose (400 rad).

Material and Method Patients with bronchogenic carcinoma scheduled to receive radiotherapy were considered for this investigation. Under study in our institution at present is the comparative therapeutic effect on bronchogenic carcinoma of daily treatments versus split weekly doses. Patients receive either

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REFERENCES

- ADERCRONBIT M. Estimation of nuclear population from microtome sections. *Anat Rec* 94 (1946) 238
- BEAUMONT H. M. The radiosensitivity of germ cells at various stages of ovarian development. *Int J Rad Biol* 4 (1962) 581
- The effects of acute X irradiation on primordial germ cells in the female rat. *Int J Rad Biol* 10 (1966) 17
- MINTZ B. Continuity of the female germ cell line from embryo to adult. *Arch Anat micr Morph exp* 48 (1959) 135
- NILSSON A. and HENRICSON B. The effect of ^{90}Sr on the ovaries of the fetal mouse. *Proc Symp Radiation Biology of the Fetal and Juvenile Mammal* 5–8 May 1969 Richland Washington USA
- OAKBERG E. I. The effect of X rays on the mouse ovary. *Genetics* 2 (1958) 207
- Irradiation damage to animals and its effect on their reproductive capacity. *J Dairy Sci* 43 (1960) 54
- Gamma ray sensitivity of oocytes of immature mice. *Proc Soc exp Biol Med* 109 (1962) 763
- Effect of 25 R of X rays at 10 days of age on oocyte numbers and fertility of female mice. *Radiation and ageing*, p. 293. *Proc of Colloquium held in Semmering Austria 1966*
- Mammalian gametogenesis and species comparisons in radiation response of the gonads. *In Effects of radiation on meiotic systems*. IAEA Vienna 1968
- PARSONS D. F. An electron microscope study of radiation damage in the mouse oocyte. *J Cell Biol* 14 (1962) 31
- PETERS H. Radiation sensitivity of oocytes at different stages of development in the immature mouse. *Radiat Res* 15 (1961) 582
- and BORUM A. The development of mouse ovaries after low dose irradiation at birth. *Int J Radiat Biol* 3 (1961) 1
- and LEVY E. Effect of irradiation in infancy on the mouse ovary: a quantitative study of oocyte sensitivity. *J Reprod Fertil* 7 (1964) 37
- RUSSELL L. B. and RUSSELL W. L. The sensitivity of different stages in oogenesis to the radiation induction of dominant lethals and other changes in the mouse. *In Progress in Radio biology*, p. 187. Oliver and Boyd Ltd Edinburgh 1956

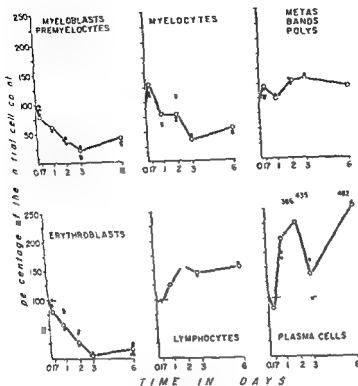


Fig 2 Marrow changes after 400 rad as a function of time expressed as a percentage of the initial count

schedule each patient had received a sternal tissue dose of 400 rad. In addition the data from the 400 rad group of the first study (examination after two days) were also included with these results. Included in this study were only patients who had no previous treatment specifically no antineoplastic drugs. Furthermore, they must have shown no demonstrable clinical laboratory or radiologic evidence of bone metastasis. In all cases studied the routine initial bone marrow aspiration revealed a bone marrow cellularity and an erythroid/myeloid cell ratio within normal limits.

Results

The marrow changes in the three groups of five patients exposed to three doses of irradiation (200 400 800 rad) and examined after two days are illustrated in Fig 1. There is a drop in the myeloblast premyelocyte group which

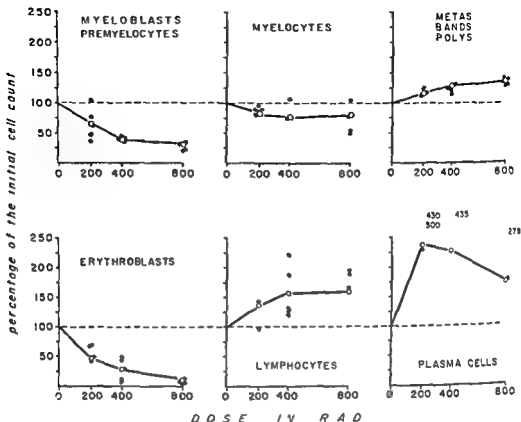


Fig 1 Relative bone marrow changes two days after localized irradiation as a function of dose. Each point represents one patient; open circles show the mean values.

daily doses (four times a week) of 200 rad or single weekly doses of 400 or 800 rad, respectively, with a three field technique, resulting in a tissue dose of 200, 400 or 800 rad $\pm 10\%$, respectively to the center of the sternum. The physical factors of the irradiation are 300 kV, 4 mm Cu HVL, 50 cm target skin distance and 48 R/min dose rate.

The investigation was divided into two separate parts. Part I was designed to investigate the effect of localized irradiation as a function of dose. Fifteen patients were included in this study: five from each dose group. Two days after the initial exposure, permission was requested for a second bone marrow aspiration, which the patients were told would be of experimental value only. In the second part of the study, which was designed to evaluate the effect of localized irradiation as a function of time, 20 additional patients, all from the 400 rad weekly split dose group, were included. Again, two aspirations were performed on each, one routinely before irradiation and another granted either 1 hour, 1, 3 or 6 days after the first set of treatments. At this point in the radiotherapy

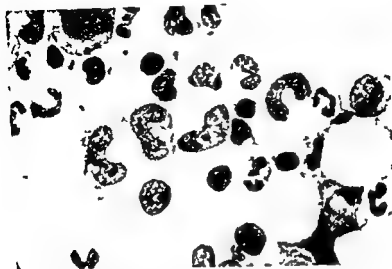


Fig 4 Giant band observed in a smear obtained 3 days after exposure to 400 rad. Complete absence of erythroblasts and presence of several lymphocytes $\times 785$

bone marrow regeneration because we also found in the 6 day smears basophilic and orthochromatic normoblasts which were not found in any of the smears obtained 2 and 3 days after irradiation. After an apparent slight initial drop at 4 hours both the lymphocytes and plasma cells later rose and remained above the initial count throughout the entire investigation (6 days). As in the first experiment the variations were wider in the plasma cells than in the lymphocytes.

No morphologic abnormalities were seen in the 4 hour smears. They were however seen in all smears taken 1, 2 and 3 days after irradiation. For the erythroblasts these were characterized by an increase in cell size, disruption of the nuclear chromatin pattern, presence of nuclear fragments in the cytoplasm and by double nuclei sometimes connected by small chromatin bridges (Fig 3). The changes in the myeloid cells were characterized by marked increase in cellular size in some of the metamyelocytes and bands (Fig 4) by the presence of toxic granulations in the cytoplasm and of finely dispersed chromatin in the nucleus and by hypersegmentation of the polymorphonuclear cells. At 6 days abnormalities were not noted in the erythroblasts but were still present in the myeloid series. No morphologic alterations could be detected at any time in the lymphocytes or plasma cells although unrecognizably distorted and damaged cells were noted in all irradiated smears. It is interesting to note that two days after irradiation there appeared to be no connection between the number of

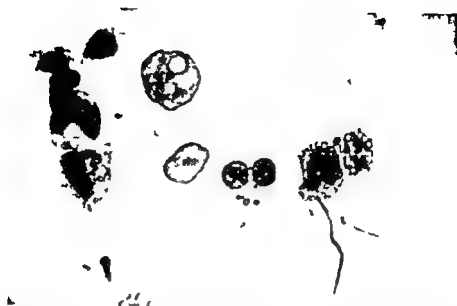


Fig. 3 Orthochromatic normoblasts have increased cell size double nucleus connected by chromatin bridges and nuclear fragments in the cytoplasm. Bone marrow obtained 2 days after exposure to 400 rad $\times 1025$

appears to be more marked at the higher dosages. There is also a drop in the mean relative percentage of the myelocytes, and a relative increase in the more mature myeloid cells the latter again appearing more marked at the two higher dosages. The most marked changes, however, were observed in the erythroblasts. The mean relative drop in these cells grew larger with the roentgen dose, the mean reaching a low point of 10.8% of the initial count after 800 rad. The relative percentage of lymphocytes rose strikingly, the mean being 137.3 for 200 rad, 157.1 for 400 rad, and 159.8 for 800 rad. The relative percentages of plasma cells followed a pattern similar to that of the lymphocytes, however they showed wider variations, due probably to the small initial count.

The changes produced by 400 rad as a function of time are recorded in Fig. 2. There was a relative drop in the myeloblast/premyelocyte group which was already evident at 4 hours, reached its lowest point at 3 days, and manifested some signs of recovery at 6 days. After an initial relative increase at 4 hours, the myelocytes followed a similar pattern but with a delay of about one day. The relative percentage of the maturing myeloid cells increased in 22 out of 25 patients and showed no clear dependency on the time of examination. The erythroblasts revealed the sharpest relative drop of any cell component. The drop began at 4 hours and as in the myeloid precursors, reached a very low point at three days (mean about 4% of the initial count) and showed some signs of recovery at 6 days. We interpreted this later increase as a sign of initial

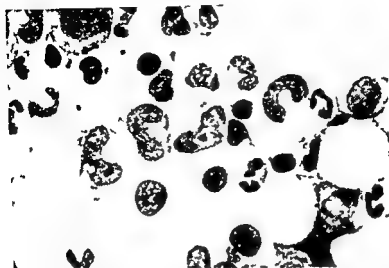


Fig 4 Giant bands observed in a smear obtained 3 days after exposure to 400 rad. Complete absence of erythroblasts and presence of several lymphocytes $\times 82$

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cells with morphologic abnormalities and the dose of irradiation received by the bone marrow

Discussion

No attempt was made to determine the absolute bone marrow count, due to the well known unreliability of such determinations on aspirated material. For this reason, although it is quite probable that all the observed percentage changes represent cell destruction, one cannot exclude the possibility of cell migration to or away from the irradiated areas. The erythroblasts and, to a lesser extent, the young cells of the myeloid series showed the most marked relative drop in the early days following irradiation. This is in the keeping with the well established radiosensitivity of these elements (DEANSTAD 1943). Moreover, the mean percentage changes in the erythroblasts seemed to be related to the dose of irradiation and to the time after irradiation, at least for the doses and times used. One can wonder whether repetitive bone marrow aspirations in the hours and immediate days after an accidental localized radiation would be of some use in approximating the degree of exposure. This of course still remains to be demonstrated.

The observation of a relative increase in the percentage of lymphocytes was quite surprising, considering the well established high radiosensitivity of the blood lymphocytes. However, this observation in humans is not unique since it has been reported after repetitive localized irradiations (GOSWITZ et coll 1963, HUTAFF & BEIDING 1955, LEHAR et coll 1966, STEWART & DISCHE 1956). Furthermore, similar findings have been described after whole body irradiation in humans and experimental animals. An increased percentage of lymphocytes was found in the bone marrow obtained after 3 to 5 weeks in humans exposed to the irradiation of the atomic bomb (OUCHTFERSON & WARREN 1956). In individual cases, there were levels as high as 80 % and most of the cells appeared to be normal small lymphocytes. An absolute increase in the marrow lymphocyte count in mice 10 days after 400 R whole body irradiation has been observed by BRECHER et coll (1948). Similarly HARRIS (1956) found an increase in the absolute number of bone marrow lymphocytes in guinea pigs exposed 10 days previously to sublethal doses of whole body irradiation. From a sequence of changes in the other bone marrow cell components, he found support for the hypothesis of MAXIMOW, who first considered the lymphocyte to be a stem cell.

The observed relative increase of lymphocytes in our experiments could be the result of repopulation of the irradiated sternal areas with normal lymphocytes migrating from non irradiated sites. According to our observations this migration if present, occurs promptly being fully manifest already one day after irradiation. Another possibility is that the bone marrow lymphocytes are relatively more

radioresistant than other bone marrow cells and also more radioresistant than the blood lymphocytes. This hypothesis could be related to the findings of STEWART & DISCHE (1956) who noted a relative lymphocytosis in the bone marrow accompanied by marked lymphopenia in the blood of six patients with ankylosing spondylitis treated 1 to 189 days previously with localized irradiation. The suggested relative radioresistance of the marrow lymphocytes would give some support to the hypothesis based on *in vitro* work, that there are two lymphocytic cell populations: one radiosensitive and one radioresistant (SCHREK & STEIANI 1964). Also this observation would have some implication in organ transplantation. Whole body irradiation has been used for suppressing the immunologic defenses of organ recipients i.e. to destroy the lymphocytes now well recognized to be the immunologic competent cell. It is obvious that if this cell is indeed more radioresistant than other bone marrow components an attempt at immunosuppression by use of radiation would be comparatively ineffective.

Acknowledgement

We wish to thank Dr Harold Schoolman for his invaluable suggestions. Dr William Neville for his kind collaboration and Mrs Zielonka and Mr Kirsh for their technical assistance.

SUMMARY

The effect of localized single doses of ionizing irradiation on normal bone marrow of patients with bronchogenic carcinoma was studied. The studies were made as a function of dose and of time. Quantitative modifications consisted of a marked percentage decrease in the erythroblasts and a less marked percentage decrease in the precursors of the granulocytic series accompanied by a shift to the right. A noticeable relative increase in lymphocytes and plasma cells starting one day post irradiation was observed.

ZUSAMMENFASSUNG

Der Effekt einer lokalisierten Einzeldosis ionisierender Strahlung auf das normale Knochenmark von Patienten mit bronchogenen Carcinomen wurde studiert. Die Untersuchungen wurden als Funktion der Dosis und der Zeit ausgeführt. Quantitative Veränderungen bestehen aus einem kräftigen prozentuellen Abfall der Erythroblasten und einem weniger ausgeprägten prozentuellen Abfall der Vorstufen der granulozytären Reihe verbunden mit einer Rechtsverschiebung. Ein bemerkenswerter relativer Anstieg der Lymphozyten und Plasmazellen der einen Tag nach der Bestrahlung einsetzte wurde beobachtet.

RÉSUMÉ

Les auteurs ont étudié l'effet des doses uniques localisées d'irradiation ionisante sur la moelle osseuse normale des malades atteints de cancer bronchique. Ces études ont été faites en fonction de la dose et du temps. Les modifications quantitatives ont consisté en une diminu-

nution importante du pourcentage des érythroblastes et en une diminution moins importante des précurseurs de la série granulocytaire accompagnée par une déviation vers la droite. Les auteurs ont observé une augmentation relative des lymphocytes et des cellules plasmocytaires commençant un jour après l'irradiation.

REFERENCES

- BRECHER C, ENDICOTT K M, GUMP H and BRAUNER H P. Effects of x ray on lymphoid and hemopoietic tissues of albino mice. *Blood* 3 (1948) 1259.
- DE NISTAD T. Radiosensitivity of bone marrow. *Acta radiol* (1943) Suppl No 52.
- GOSWITZ F A, ANDREWS G A and KMSLETA R M. Effect of local irradiation (Co^{60} teletherapy) on the peripheral blood and bone marrow. *Blood* 21 (1963) 605.
- HARRIS I I. Quantitative examination of bone marrow in guinea pigs after gamma irradiation. *Brit med J* 1956 II p 1032.
- HUTAFF L W and BELDING H W. Effects of irradiation of pelvis in patients with carcinoma of the cervix uteri on iliac and sternal marrow and peripheral blood. *Amer J Roentgenol* 73 (1955) 251.
- LEHAR T J, KIELY J M, PEASE G L and SCANLON P W. Effect of focal irradiation on human bone marrow. *Amer J Roentgenol* 96 (1966) 183.
- OUCHTERSON A W and WARREN S (Editors). Medical effects of the atomic bomb in Japan. McGraw Hill New York 1956.
- SCHIREK R and STEFANI S. Radioreistance of phytohemagglutinin treated normal and leukemic lymphocytes. *J nat Cancer Inst* 23 (1964) 507.
- STEWART J W and DISCH S. Effect of radiotherapy on bone marrow in ankylosing spondylitis. *Lancet* 1956 II p 1063.
- SIXES M P, CHU F C H and WILKERSON W G. Local bone marrow changes secondary to therapeutic irradiation. *Radiology* 75 (1960) 919.
- — SAEEL H et coll. The effects of varying dosages of irradiation upon sternal marrow regeneration. *Radiology* 83 (1964) 1084.

DEPIGMENTATION OF HAIR IN TWO STRAINS OF MICE AFTER SINGLE AND FRACTIONATED DOSES OF ROENTGEN RAYS

by

E. V. HULSE and L. G. MIZON

Depigmentation of hair has usually been studied as a response occurring a few weeks after irradiation and experiments have entailed plucking hair to synchronise hair cycles and often to measure the radiation response (CHAST 1948 1949 POTTEN 1968 POTTEN & HOWARD 1969). The present experiments using roentgen rays were intended to supplement observations on the effects of beta irradiation of mouse skin (HULSE 1967 HULSE & MOLE 1969 HULSE DEMPSEY & BATCHELOR unpublished) and were designed to follow the process of depigmentation over several months with as little interference as possible. Consequently hairs were not plucked out at any stage and apart from the irradiations hair growth was allowed to proceed naturally. Part body irradiation was used to avoid any general effects.

Methods

Mice Female CBA/H and C57Bl/H mice aged 6 to 9 months were randomized and kept 4 to a cage. The numbers of mice used at each dose level and type of fractionation are given in the Table.

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Table

Number of mice exposed to single doses or two equal fractions of various doses of roentgen rays and the time between the fractions

Strain	Total dose (rad)	Number of fractions	Time between fractions (hours)	Number of mice irradiated	Number of mice alive at 48 weeks
CBA	500	1	—	16	14
	500	2	6	16	16
	500	2	24	16	15
	250	1	—	16	15
C57Bl	750	1	—	4	3
	500	1	—	8	8
	500	2	6	8	8
	500	2	24	8	7
	400	1	—	4	4
	250	1	—	8	8

Irradiations A 2 cm transverse zone across the middle of the torso was irradiated. The mice were exposed in separate perspex tubes, 2.5 cm internal diameter, with perforations at one end to allow a plentiful supply of fresh air. The closures of the tube, at the tail end, were adjusted so that the mouse was kept in one position during the irradiation. The mice were irradiated dorso-ventrally, four at a time, and, except for the 2 cm gap over the mid torso, were shielded by lead sheets 6 mm thick. The irradiation factors were 250 kV, 135 rad/min with a HVL of 1.2 mm Cu.

Scoring the degree of depigmentation The mice were examined weekly for 48 weeks and the degree of depigmentation of the dorsal aspect of the irradiated zone was judged from the general overall naked eye appearances. The system of scoring was originally evolved to study effects of beta irradiation (Hulse et al., unpublished). Normal hair without any depigmentation scored zero. An irradiated zone in which the hair was completely white without any evidence of natural colour scored 8. Intermediate stages received appropriate scores in the range 1 to 7, e.g. when careful examination revealed a few depigmented hairs in the irradiated zone a score of 1 was given; when the colour of the irradiated zone appeared about half way between normal and complete depigmentation the score was 4; and when only a few scattered pigmented hairs prevented the zone being judged as completely white a score of 7 was

given. Weekly means and standard errors were calculated for each irradiation group.

In order to ensure that the scoring was consistent animals which had received the lower doses were always examined first. A few scattered white hairs are normally present in C57Bl/H mice and these were disregarded. The unirradiated skin providing a standard for judging their incidence. Neither strain spontaneously develops depigmented hair during the age range covered by this experiment.

Results

CBA mice (Fig 1) After a single dose of 500 rad depigmentation was first noticed at 7 weeks after irradiation. It progressed steadily to 23 weeks and then remained more or less unchanged until 36 weeks when a further slight increase in depigmentation started. A single dose of 250 rad did not produce any discernible depigmentation.

When 500 rad was given in two fractions the onset of visible depigmentation was postponed and the degree of depigmentation was consistently less. The 24 hour interval between doses reducing the effect and postponing the onset more than the 6 hour interval. From 25 to 27 weeks onwards the degree of depigmentation remained steady until a second minor increase occurred at 40 weeks after the 6 hour interval and at 47 weeks after the 24 hour interval.

C57Bl mice (Fig 2) After single doses of 400 to 750 rad the degree of depigmentation, its rate of development and its time of onset were dose dependent. Again no depigmentation occurred after 250 rad. Dividing 500 rad into two fractions delayed the onset and reduced the degree of depigmentation below that which occurred after a single dose of 400 rad. A 24 hour interval between fractions again reduced the effect more than a 6 hour interval. The degree of depigmentation remained static from 25 to 30 weeks onwards in all groups except those given a single dose of 400 rad which showed a slight but non significant increase from 43 to 46 weeks.

Discussion

The present investigation differs from those of previous workers in the following ways: (1) measurements of depigmentation were rapid and did not require any special kill or apparatus; (2) the mice were irradiated when several months older than usual, e.g. 4 to 8 months older than POTTEKENS (1968); and (3) measurements for the dose response curve (Fig 3) were taken almost one year after irradiation rather than at a few days or weeks.

The response of the hair follicle to radiation varies according to the stage of the hair cycle at the time of exposure. Depigmentation is greater when resting

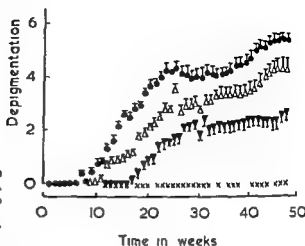


Fig. 1. Average weekly depigmentation scores with standard errors for CBA mice. Single doses of 500 rad (●), two doses of 250 rad with a 6 hour interval (△) and with a 24 hour interval (▽), single dose of 250 rad (×).

(telogen) hair is exposed and delay in hair growth is greater when growing (anagen) hair is irradiated (CHASE 1949). However, variations in sensitivity during the hair cycle are not important in the present investigation. The hair of mice aged 6 to 9 months is in the resting phase most of the time and the likelihood of irradiating unplucked hair follicles during anagen is very slight; indeed, CHASE (1949) found that depigmentation following the irradiation of unplucked follicles was the same as that following the irradiation of follicles known to be in telogen. Post irradiation delay in hair growth would be minimal in resting hairs and our observations extended over many months. Thus, any temporary delay in hair growth did not interfere with the assessment of final depigmentation, which in most cases was stationary over several weeks.

The present observations (Figs 1 and 2) confirm that hair depigmented by ionizing radiation does not regain its colour, even after many months (DANEI & LUBNOW 1936, CHASE 1949). The second increase in depigmentation seen in CBA mice at 40 weeks after irradiation (Fig. 1) corresponds to that seen in F_1 (C57L \times A/He) mice about one year after exposure to gamma rays from an atom bomb (URTON *et al.* 1960). The absence of a second increase in depigmentation in C57Bl mice (Fig. 2) is in agreement with CHASE'S (1949) observations.

Sensitivity of C57Bl and CBA mice. Apart from the difference in the delayed increase in depigmentation, the response of the two strains was very similar, e.g. at 40 weeks the scores for 500 rad given as a single dose or as two fractions with a 6 hour or a 24 hour interval were about 5.3 and 2 respectively in both strains (Figs 1 and 2). This confirms that strain differences are slight after a single dose (POTTEN 1969) and also shows that the same applies for depigmentation after fractionation.

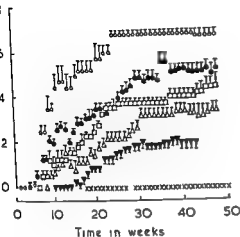


Fig. 2. Average weekly depigmentation scores with standard errors for C57Bl mice: ■ single dose of 750 rad ○ of 500 rad ● of 400 rad □ two doses of 250 rad with a 12 hour interval △ and with a 24 hour interval ▼ single dose of 250 rad × (Data on two doses of 250 rad with a 24 hour interval cease at 43 weeks because three of the remaining seven mice developed generalised alopecia which is not uncommon in aged C57Bl/H mice. The degree of depigmentation did not alter in the remaining mice of the group.)

Threshold dose Irradiation with 250 rad was below the threshold for hair depigmentation in CBA and C57Bl mice (Figs 1 and 2) and similar threshold values have been obtained previously in C57Bl and in F_1 (C57L \times A/He) mice (CHASE 1949 UPTON et coll 1960). Each hair follicle is supplied with a number of melanocytes and the finding of a threshold dose for the depigmentation of the hair does not imply that no melanocytes whatsoever are permanently affected by lower doses. Nevertheless there were only minimal changes in counts of hair follicle melanocytes after 250 rad of 300 kV roentgen rays (POTTEN 1968).

Survival curves In the present investigation depigmentation was judged by assessing the colour of the irradiated zone and no attempt was made to count depigmented hairs or changes in the number of melanocytes. Nevertheless the data can be used to obtain a dose response curve by subtracting the final score of a group of mice from the maximum possible (i.e. 8) and expressing this as a fraction of 8. The resulting type C survival curve for C57Bl mice is given in Fig. 3. The D_{37} is 260 rad and the extrapolation number 2.5. These values are similar to those for resting hairs obtained by more refined techniques. Measurements of the mean number of melanocytes per follicle gave $D_{37} = 180$ –220 rad (POTTEN 1968) and counts of the number of follicles with one or more melanocytes gave $D_{37} = 346$ –656 rad and $n = 2$ (POTTEN 1968 POTTEN & HOWARD 1969). CHASE (1949) did not construct survival curves but his data on resting hairs can be dealt with in the same way as our data (Fig. 3). For 200 kV roentgen rays $D_3 = 300$ R and $n = 2$ and for 100 kV roentgen rays $D_3 = 280$ R and $n = 2.5$.

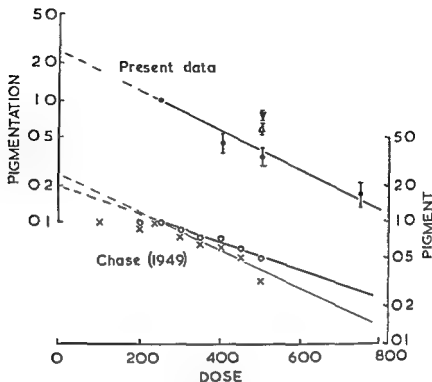


Fig 3 *Upper curve and left hand ordinate scale* Survival curve for hair colour remaining in C57B1 mice at the end of present experiment (doses in rad) Single doses ● two doses of 250 rad with a 6 hour interval Δ and with a 24 hour interval ▼

Lower curves and right hand ordinate scale Survival curves for hair colour remaining in CHASE's (1949) C57B1 mice irradiated during the resting phase of hair growth (appropriate data calculated from Tables 1 and 2 of CHASE (1949) with doses in R) ○ denotes 200 kV roentgen and × 100 kV roentgen radiation

Fractionation Reported exposures to subthreshold doses produced depigmentation (DANEEI & IUBNOW 1936, CHASE & RAUCH 1950). In the present experiments two subthreshold exposures, to either strain produced depigmentation. In no case was the effect as great as it would have been if the two doses had been given consecutively without a radiation free interval. However, the amount of depigmentation was greater with the shorter interval between doses (Figs 1 and 2).

As read from the graph (Fig 3) the single dose (D_1) which would have produced the same effect as 500 rad given as two equal fractions (D) is 390 rad for a 6 hour interval between fractions and 360 rad for a 24 hour interval. Thus $(D - D_1)$ 6 hours = 110 rad and $(D - D_1)$ 24 hours = 140 rad. CHASE (1948) found that $(D - D_1)$ 3 days = 200 R.

Acknowledgements

We are very grateful to Mr and Mrs M. J. Corp for the irradiations

SUMMARY

Depigmentation of hair after part body irradiation of mice was judged visually at weekly intervals. The onset rate of increase and final degree of depigmentation at 25 to 40 weeks were dose dependent. The threshold was about 250 rad. Dividing a dose into two equal fractions decreased the degree of depigmentation: a 24 hour interval was more effective than one of 6 hours. The observations allowed values for melanocyte survival to be deduced ($D_{37} = 260$ rad, $n = 2.5$). For fractionated doses D_0-D_1 was 110 to 140 rad.

ZUSAMMENFASSUNG

Die Depigmentierung der Haare nach Teilkörperbestrahlung von Mäusen wurde visuell in wöchentlichen Intervallen festgestellt. Der Beginn, die Geschwindigkeit des Anstiegs und der abschließende Grad der Depigmentierung zwischen 25 und 40 Wochen sind dosisabhängig. Die Schwelle liegt über 250 rad. Teilt man die Dosis in zwei gleiche Fraktionen, sinkt der Grad der Depigmentierung: ein 24 Stunden Intervall ist effektiver als ein 6 Stunden Intervall. Die Beobachtungen erlauben Überlebenswerte für die Melanozyten herzuleiten ($D_{37} = 260$ rad, $n = 2.5$). Für fraktionierte Dosen war D_0-D_1 110 bis 140 rad.

RÉSUMÉ

Les auteurs ont apprécié visuellement à intervalles hebdomadaires sur deux souches de souris la dépigmentation des poils après irradiation d'une partie du corps. Le début, la vitesse d'accroissement et le degré final de dépigmentation entre 25 et 40 semaines dépendent de la dose. Le seuil est au dessus de 250 rad. La division de la dose en deux fractions égales diminue le degré de dépigmentation: un intervalle de 24 heures a plus d'effet qu'un intervalle de 6 heures. Les observations ont permis de déduire des valeurs de survie des melanocytes ($D_{37} = 260$ rad, $n = 2.5$). Pour les doses fractionnées D_0-D_1 est compris entre 110 et 140 rad.

REFERENCES

- CHASE, H. B. Time factor with respect to X-ray induced greying in the mouse. *Genetics* 33 (1948) 100.
 CREYNO, of hair. I. Effects produced by single doses of X-rays on mice. *J. Morph.* 84 (1949) 7.
 — and RALPH, H. Greying of hair. II. Response of individual hairs in mice to variations in X-radiation. *J. Morph.* 87 (1950) 481.
 DANIEL, R. und LEBSON, E. Zur Physiologie der Kälteschwarzung beim Russenkaninchen. II. Der Einfluss von Röntgenstrahlung auf die Premalbildung. *Biol. Zbl.* 56 (1936) 472.
 HULSE, E. A. Incidence and pathogenesis of skin tumours in mice irradiated with single doses of low energy beta particles. *Brit. J. Cancer* 21 (1967) 531.

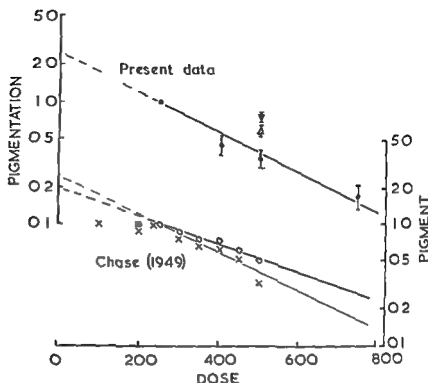


Fig. 3 *Upper curve and left hand ordinate scale*: Survival curve for hair colour remaining in C57Bl mice at the end of present experiment (doses in rad). Single doses ● two doses of 250 rad with a 6 hour interval Δ and with a 24 hour interval ∇

Lower curves and right hand ordinate scale: Survival curves for hair colour remaining in CHASE's (1949) C57Bl mice irradiated during the resting phase of hair growth (appropriate data calculated from Tables 1 and 2 of CHASE (1949) with doses in R). ○ denotes 200 kV roentgen and × 100 kV roentgen radiation

Fractionation Reported exposures to subthreshold doses produced depigmentation (DANEFL & LUBNOW 1936 CHASE & RAUGH 1950). In the present experiments two subthreshold exposures, to either strain, produced depigmentation. In no case was the effect as great as it would have been if the two doses had been given consecutively, without a radiation free interval. However, the amount of depigmentation was greater with the shorter interval between doses (Figs 1 and 2).

As read from the graph (Fig. 3) the single dose (D_1) which would have produced the same effect as 500 rad given as two equal fractions (D) is 390 rad for a 6 hour interval between fractions and 360 rad for a 24 hour interval. Thus $(D - D_1)$ 6 hours = 110 rad and $(D - D_1)$ 24 hours = 140 rad. CHASE (1948) found that $(D - D_1)$ 3 days = 200 R.

CARCINOMES EPIDERMOIDES DE LA LANGUE MOBILE ET DU PLANCHER BUCCAL

Etude de 245 cas traites à l'Institut Gustave Roussy

par

B PIERQUIN, D CHASSAGNE, Y CACHIN, F BAILLET et F FOURNELLE LE BUIS

Notre étude porte sur 245 cas de carcinomes épidermoïdes de la langue mobile et du plancher buccal (T1, T2 et T3) traités entre le début de 1960 et la fin de 1965. Nous disposons donc d'un recul d'observation minimale supérieur à 3 ans.

Le but principal de ce travail est d'apprécier les résultats de l'endocurietherapie par iridium 192 avec nos nouvelles techniques de préparation non radio active en les comparant aux résultats de la technique classique d'endocurietherapie par radium (BAILLET 1968).

Données cliniques Nous avons regroupé tous les cas de carcinomes épidermoïdes intéressant les muqueuses des parties molles « situant à l'intérieur de l'arc mandibulaire » nous associons donc les lésions de la langue mobile et celles du plancher buccal en excluant les localisations tumorales débordant dans la base de langue sur plus d'un centimètre d'un part envahissant directement la muqueuse gingivale d'autre part. Pratiquement cette délimitation revient à étudier tous les cancers intéressant exclusivement les parties molles comprises dans l'arc mandibulaire ce qui revient à éliminer les tumeurs marginales d'une

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- and MOLE R. H. Skin tumour incidence in CBA mice given fractionated exposures to low energy beta particles *Brit J Cancer* 23 (1969) 452
- POTTEN C. S. Radiation depigmentation of mouse hair: a study of follicular melanocyte populations *Cell Tissue Kinet* 1 (1968) 239
- Radiation depigmentation of mouse hair: effects of mouse strain *Brit J Derm* 81 (1969) 289
- and HOWARD A. Radiation depigmentation of mouse hair: the influence of local tissue oxygen tension and radiosensitivity *Radiat Res* 38 (1969) 65
- UPTON A. C. KIMBALL A. W. FURTH J. et coll. Some delayed effects of atom bomb radiations in mice *Cancer Res* 20 (1960) No 8 part 2

Tableau I

Repartition des cas (au total 245) selon le systeme TNM

	N0	N1	N2	N3	Total
T1	III	2			20
T2	68	33	7	11	119
(T2a)	(30)	(14)	(5)	(7)	(51)
(T2b)	(38)	(19)	(2)	(9)	(68)
T3	48	31	8	19	106
(T3)	(27)	(19)	(3)	(7)	(56)
(T3b)	(21)	(12)	(5)	(12)	(50)
Total	134	66	15	30	245

des tumeurs primitives ainsi que leurs sous-groupes (T2 et T3) se repartissent en nombres a peu pres equivalents d'autre part que les adenopathies cliniquement apparentes avant le traitement sont dans les memes groupes et sous groupes de l'ordre de 50 %

Traitement

Conduite generale pour les malades des groupes T1 T2 et T3a Le traitement se compose (1) d'une endocurietherapie de la tumeur primitive (radium ou iridium 192) dans un premier temps (2) en cas d'adenopathie cliniquement palpable d'une chirurgie avec curage ganglionnaire sous-maxillaire bilaterale et jugulo-carotidien uni ou bilaterale en fonction de l'extension metastatique uni ou bilaterale au niveau du ganglion de Lutner (3) en cas d'adenopathie histologiquement confirmee (N+) d'une telerradiotherapie des aires lymphatiques cervicales droite et gauche (3 500 rad pour les cas traites par tele roentgentherapie sous 200 kV 5 000 a 6 000 rad pour les cas traites par telecobalt)

Si aucune adenopathie n'est cliniquement perceptible le traitement se limite a l'endocurietherapie de la tumeur primitive une surveillance reguliere des aires lymphatiques s'exerce ensuite tous les deux ou trois mois si une adenopathie secondaire apparait le curage chirurgical suivi de l'eventuelle telerradiotherapie sont alors aussitot appliques

Conduite generale pour les malades du groupe T3b Ce protocole therapeutique est modifie qu'en fonction de la tumeur primitive du fait de l'importance de l'extension tumorale une telerradiotherapie par telecobalt precede l'endocurietherapie (4 500 rad) l'endocurietherapie ne s'applique qu'au reliquat tumoral (4 000 rad)

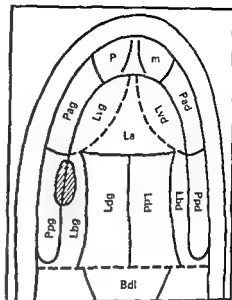


Fig 1 Schéma topographique de la langue et du plancher. Exemple d'un carcinome épidermoïde pelvi lingual (Ppg et Lbg T2a)

Pm — plancher médian
Pag — Lad — plancher antérieur gauche ou droit
Ppg — Ppd — plancher postérieur gauche ou droit
La — langue antérieure
Lg — Ld — langue ventrale gauche ou droite
Lbg — Lbd — bord de langue gauche ou droit
Ldg — Ldd — langue dorsale gauche ou droite
Bdl — base de langue

part, les très grosses tumeurs débordant sur les structures voisines d'autre part (T4)

Tous nos cas sont des lésions neuves, sans traitement local antérieur, sans autre localisation tumorale simultanément associée. Sur ces 245 cas, nous dénombrons 230 hommes et 15 femmes. La moyenne d'âge est de 57 ans. Dans la très grande majorité des cas, il s'agit de sujets tics, éthyliques et disposant d'une mauvaise dentition.

Nous avons classé l'extension locale et ganglionnaire selon le système TNM, à savoir, pour la tumeur primitive

T1 — tumeur de moins de 1 cm, sans infiltration sous muqueuse palpable,
T2 — tumeur entre 1 et 3 cm, avec infiltration sous muqueuse inférieure à 1 cm,

T3 — tumeur entre 3 et 5 cm, avec une infiltration inférieure à 3 cm
et pour les adénopathies

N0 — pas d'adénopathie palpable,
N1 — adénopathie(s) mobile(s) et homolatérale(s),
N2 — adénopathie(s) mobile(s) contralatérale(s) ou bilatérale(s),
N3 — adénopathie(s) fixée(s)

Nous avons adjoint, pour la tumeur primitive, une étude topographique basée sur un quadrillage délimitant 13 secteurs (voir figure 1)

Cette étude topographique jointe à celle de l'extension nous a permis de définir deux sous-groupes pour les T2 et les T3 : T2a, T2b, T3a, et T3b

La répartition de nos 245 cas selon le système TNM est donnée sur le Tableau 1. On constate d'une part que les deux groupes les plus importants

Tableau 3

Distribution de la survie des cas en fonction de l'extension locale et ganglionnaire (normalisee a 3 ans d'observation)

	N0	N1	N2	N3	Total	
T1	14/18	2/2			16/20	(80 %)
T2	31/68	12/33	4/7	3/11	50/119	(42 %)
T3	27/48	14/31	3/8	7/19	46/106	(43 %)
Total	67/134 (50 %)	28/66 (42 %)	7/15 (46 %)	10/30 (33 %)	112/245	(46 %)

avec tubes plastiques et fils radio-actifs (fils d'iridium 192 de 0.3 mm de diamètre). Ces techniques ont été décrites dans d'autres travaux (PIERQUIN 1964 1969).

Grosso modo la technique par gouttières vectrices est réservée aux tumeurs T1 T2 et T3a cependant que la technique par tubes plastiques est réservée aux tumeurs T3b. La dose tumorale calculée par la technique sur points (PIERQUIN et coll 1962) est de 7 000 rad dans les cas traités par la seule endocurietherapie de 4 000 rad dans les cas traités par association teleradiotherapie endocurietherapie cette dose tumorale est calculée sur une isodose de référence dont la valeur se situe entre 80 et 90 % de celle de la dose de base. Le débit quotidien est de l'ordre de 1 000 à 1 500 rad ce qui donne pour une dose totale de 7 000 rad une durée d'application de 5 à 7 jours.

La répartition des cas traités soit par radium soit par iridium 192 en fonction de l'extension tumorale est présentée sur le Tableau 2.

On constate une répartition très différente de ces deux modalités d'endocurietherapie en fonction de l'extension tumorale. Grosso modo les indications de l'endocurietherapie par radium ont intéressé des lésions moins étendues que celles traitées par iridium 192.

Resultats

La survie de nos 245 cas est normalisée à 3 ans d'observation. Sa distribution en fonction de l'extension locale et ganglionnaire apparaît sur le Tableau 3.

Ces taux de survie font apparaître trois faits : (1) le taux de survie des T1 est très élevé (80 %) la plupart des T1 ne présentent pas d'adenopathie au moment du traitement ; (2) les taux de survie des T2 et des T3 sont identiques entre 1 et 5 cm les carcinomes épidermoïdes de la langue mobile et du plancher buccal ont un pronostic identique ; (3) la présence ou l'absence d'adenopathie clinique apparente au moment du traitement ne modifie pas fondamentalement

Tableau 2

Repartition des cas traités soit par radium soit par iridium 192 en fonction de l'extension tumorale

	Radium	Iridium	Pas d'endocurie	Total
T1	17	2	1	20
T2	90	27	1	119
(T2a)	(44)	(7)	(0)	(51)
(T2b)	(46)	(20)	(2)	(68)
T3	32	66	8	106
(T3a)	(22)	(32)	(2)	(56)
(T3b)	(10)	(34)	(6)	(50)
Total	139	95	11	245

Modalités de l'endocurietherapie L'ensemble de nos malades a été traité selon deux modalités d'endocurietherapie : (1) utilisant les aiguilles de radium, et (2) utilisant des fils d'iridium 192.

1 Il s'agit d'une technique classique utilisant des aiguilles d'assez faibles longueurs radio-actives (entre 0,6 et 1,7 cm) avec une activité linéaire de l'ordre de 1,33 mCi/cm. L'implantation s'effectue sous anesthésie générale. La disposition du matériel radioactif reste habituellement parallèle à la surface muqueuse en cas de lésion non infiltrante, perpendiculaire à cette surface en un ou deux plans en cas de lésion infiltrante. Pour des lésions centrées autour du sillon palatin lingual, l'endocurietherapie s'effectue le plus souvent en deux temps (un temps lingual et un temps palatin) successifs, dans tous les autres cas, en un seul temps. Pour les lésions du plancher buccal, un appareillage de contention en matière plastique avec appui sur la mandibule et fixation par suture cutanée maintient le dispositif radioactif en place (technique de H. Kutter VIGNIER 1961).

La dosimétrie reste basée sur des règles empiriques, basées sur l'expérience clinique et calculée en milligrammes heures. Un certain nombre de contrôles ont permis de constater d'assez grandes variations dans la dose tumorale d'un cas à un autre. Grosso modo, cette dose est très élevée dans les petits volumes cibles (T1 et T2a), de l'ordre de 10 000 rad, relativement faible et surtout inhomogène dans les volumes cibles de moyenne importance (T2b et T3), de l'ordre de 5 000 à 6 000 rad. La durée d'application est, le plus souvent, de six jours.

2 Avec l'endocurietherapie par fils d'iridium 192 il s'agit de deux modalités techniques avec préparation non radio active, l'une avec gouttières vectrices et épingle radio active (fils d'iridium 192 de 0,5 mm de diamètre), l'autre

Tableau 5

Comparaison entre les résultats des deux modalités d'endocurietherapie par la fréquence des récurrences (ou des non stérilisations) de la tumeur primitive

	Radium	Ir 192
T1	3/17 (17 %)	0/9
T2	30/90 (33 %)	1/27 (4 %)
(T2a)	(13/44)	(0/7)
(T2b)	(17/46)	(1/20)
T3	14/39 (43 %)	11/61 (16 %)
(T3)	(9/22)	(2/32)
(T3b)	(5/10)	(7/34)

Il existe donc une différence modérée en faveur de l'iridium 192 où le taux de survie pour les T2 et les T3 atteint 50 % alors qu'il ne dépasse pas 40 % avec le radium. Nous discuterons plus loin de la signification de cette différence.

Une comparaison plus précise entre les résultats de ces deux modalités d'endocurietherapie peut être établie en étudiant la fréquence des récurrences (ou des non stérilisations) de la tumeur primitive observée à 3 ans. Le Tableau 5 chiffre ces taux de récurrences.

Les différences dans la fréquence des récurrences entre radium et iridium 192 sont très importantes. Pour les T2, les récurrences avec l'iridium 192 sont huit fois moins fréquentes. Pour les T3, elles sont trois fois moins fréquentes. Cette différence est encore plus frappante si l'on compare les malades qui n'ont subi que la seule endocurietherapie, c'est-à-dire les malades des groupes T1, T2 et T3a (lésions de moins de 4 cm de longueur et de 2 cm d'infiltration) : on observe les taux suivants :

	Radium	Ir 192
T1 T2 T3a	42/129	3/61
Taux bruts	33 %	5 %
Taux pondérés	35 %	3 %

Une conclusion s'impose pour les cancers de la langue mobile et du plancher buccal : les récurrences sont dix fois moins fréquentes au niveau de lésions de moins de 4 cm lorsque l'on utilise l'iridium 192 en préparation non radio-active, selon une dosimétrie bien contrôlée, au lieu du radium selon une dosimétrie empirique.

Résultats en fonction des adénopathies La survie en fonction des N0, N1, N2 et N3 n'accuse pas de différences très importantes. Mais ces résultats appa-

Tableau 4

Les taux de survie à 3 ans en fonction du traitement de la tumeur primitive

	Radium	Iridium	Pro d'endocurie	Total
T1	14/17	1/2	1/1	16/20
T2	37/90	13/27	0/2	50/119
(T2a)	(21/44)	(4/7)		(25/51)
(T2b)	(16/46)	(9/20)	(0/2)	(25/68)
T3	14/32	32/66	0/8	46/106
(T3a)	(11/22)	(18/32)	(0/2)	(29/56)
(T3b)	(3/10)	(14/34)	(0/6)	(17/50)
Total	63/139	46/93	1/11	110/245

le pronostic (si l'on met à part les T1, le pronostic des N0 est identique à celui des N1 et des N2, pour T2 et les T3)

Il convient d'étudier plus en détail ces résultats globaux, en fonction du traitement de la tumeur primitive d'un part, et en fonction des adénopathies d'autre part

Résultats en fonction du traitement de la tumeur primitive Les taux de survie à 3 ans, en fonction du traitement de la tumeur primitive apparaissent sur le Tableau 4

Globalement, ces taux de survie font apparaître des résultats identiques entre radium et iridium. Mais la répartition des deux techniques entre les T1, les T2 et les T3 est trop inégale pour tirer une conclusion valable à partir de ces totaux. Ceci est particulièrement évident pour les T1 qui ont été presque tous traités par radium et dont les conditions cliniques et pronostiques apparaissent très différentes (adénopathies exceptionnelles et taux de survie très élevés)

Un essai de comparaison entre la survie des cas traités par radium et ceux traités par iridium 192 ne peut être tenté que pour les T2 et les T3 dont les conditions cliniques et pronostiques sont très comparables. En reprenant les résultats du Tableau 4 pour les T2 et les T3, on obtient les taux suivants

	Radium	Iridium
Taux bruts	42 %	48 %
Taux pondérés	40 %	50 %

Les taux pondérés sont obtenus en supposant que tous les malades (T2a, T2b, T2c et T3b) ont été traités soit par radium, soit par iridium 192

Tableau 5

Comparaison entre les résultats des deux modalités d'endocurietherapie par la fréquence des récurrences (ou des non stérilisations) de la tumeur primitive

	Radium	Iridium
T1	3/17 (17 %)	0/2
T2	30/90 (33 %)	1/27 (4 %)
(T2a)	(13/44)	(0/7)
(T2b)	(17/46)	(1/20)
T3	14/32 (43 %)	11/16 (68 %)
(T3a)	(9/22)	(2/12)
(T3b)	(5/10)	(7/4)

Il existe donc une différence modérée en faveur de l'iridium 192 où le taux de survie pour les T2 et les T3 atteint 50 % alors qu'il ne dépasse pas 40 % avec le radium. Nous discuterons plus loin de la signification de cette différence.

Une comparaison plus précise entre les résultats de ces deux modalités d'endocurietherapie peut être établie en étudiant la fréquence des récurrences (ou des non stérilisations) de la tumeur primitive observée à 3 ans. Le Tableau 5 chiffre ces taux de récurrences.

Les différences dans la fréquence des récurrences entre radium et iridium 192 sont très importantes : pour les T2 les récurrences avec l'iridium 192 sont huit fois moins fréquentes ; pour les T3 elles sont trois fois moins fréquentes. Cette différence est encore plus frappante si l'on compare les malades qui n'ont subi que la seule endocurietherapie c'est à dire les malades des groupes T1, T2 et T3a (lésions de moins de 4 cm de longueur et de 2 cm d'infiltration) : on observe les taux suivants :

	Radium	Iridium
T1 T2 T3a	42/129	3/61
Taux bruts	33 %	5 %
Taux pondérés	35 %	3 %

Une conclusion s'impose : pour les cancers de la langue mobile et du plancher buccal les récurrences sont dix fois moins fréquentes au niveau de lésions de moins de 4 cm lorsque l'on utilise l'iridium 192 en préparation non radio-active selon une dosimétrie bien contrôlée au lieu du radium selon une dosimétrie empirique.

Résultats en fonction des adénopathies La survie en fonction des N0, N1, N2 et N3 n'accuse pas de différences très importantes. Mais ces résultats appa-

Tableau 4

Les taux de survie à 3 ans en fonction du traitement de la tumeur primitive

	Radium	Iridium	P ₁₅ d'endocruc	Total
T1	14/17	1/2	1/1	16/20
T2	37/90	13/27	0/2	50/119
(T2a)	(21/44)	(4/7)		(25/51)
(T2b)	(16/46)	(9/20)	(0/2)	(25/68)
T3	14/32	32/66	0/8	46/106
(T3a)	(11/22)	(18/32)	(0/2)	(29/56)
(T3b)	(3/10)	(14/34)	(0/6)	(17/50)
Total	65/139	16/95	1/11	112/245

le pronostic (si l'on met à part les T1, le pronostic des N0 est identique à celui des N1 et des N2, pour T2 et les T3)

Il convient d'étudier plus en détail ces résultats globaux, en fonction du traitement de la tumeur primitive d'un part, et en fonction des adénopathies d'autre part

Résultats en fonction du traitement de la tumeur primitive Les taux de survie à 3 ans, en fonction du traitement de la tumeur primitive apparaissent sur le Tableau 4

Globalement, ces taux de survie sont apparus des résultats identiques entre radium et iridium. Mais la répartition des deux techniques entre les T1, les T2 et les T3 est trop inégale pour tirer une conclusion valable à partir de ces totaux. Ceci est particulièrement évident pour les T1 qui ont été presque tous traités par radium et dont les conditions cliniques et pronostiques apparaissent très différentes (adénopathies exceptionnelles et taux de survie très élevés)

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	Radium	Iridium
Taux bruts	42 %	48 %
Taux pondérés	40 %	50 %

Les taux pondérés sont obtenus en supposant que tous les malades (T2a, T2b, T3a, et T3b) ont été traités soit par radium soit par iridium 192

Trois faits apparaissent (1) la proportion des curages négatifs (N—) est relativement importante (39/129 soit 30 %) (2) la répartition et la survie des N+ et des N— est sensiblement équivalente entre les Np1 et les Np2 (3) le pronostic des curages négatifs (N—) est bon et s'identifie à celui des N0 restes N0 74 % de survie à 3 ans pour les N— et 72 % pour les N0 restes N0

Parmi les curages positifs (N+) nous avons étudié le potentiel extensif des métastases ganglionnaires en observant l'état de la capsule enveloppant les ganglions métastatiques. Nous avons regroupé les cas avec capsule intacte (C—) et ceux avec capsule envahie ou rompue (C+) par le processus épithélio-mateux. En voici la survie à 3 ans

N +	C +		C —		Total	
Np1	2/25	(8 %)	14/29	(48 %)	16/54	(29 %)
Np2	6/27	(22 %)	6/9	(66 %)	12/36	(33 %)
Total	8/52	(15 %)	20/38	(53 %)	28/90	(31 %)

Deux faits apparaissent

1 La proportion des capsules rompues est légèrement minoritaire parmi les Np1 (25/54 46 %) elle devient fortement majoritaire parmi les Np2 (27/36 75 %). La fréquence des capsules rompues (C+) s'accroît donc avec le temps.

2 Le pronostic des curages positifs (N+) est fondamentalement déterminé par l'état de la capsule. Il est très sombre parmi les cas avec capsule rompue (15 % de survie à 3 ans) avec cependant un meilleur pronostic parmi les Np2. Il reste bon parmi les cas avec capsule intacte (53 % de survie à 3 ans) avec un taux légèrement supérieur à la moyenne de survie globale (53 %) contre 46 %.

Nous avons enfin cherché à apprécier le rôle de la téléradiothérapie (téléroentgénothérapie sous 200 kV ou télécobalt) des aires lymphatiques sur la survie à 3 ans. Parmi nos 90 malades avec curage positif (54 Np1 + 36 Np2) un certain nombre ont subi une irradiation lymphatique supérieure à 5 000 rad (54 cas) cependant que les autres n'ont pas été irradiés (surtout parmi les Np2) ou ont subi une irradiation inférieure à 4 500 rad (36 cas). Les résultats de la survie à 3 ans pour ces deux groupes sont les suivants

Téléradiothérapie de 0 à 4 500 rad	7/36	(19 %)
Téléradiothérapie plus de 5 000 rad	21/54	(39 %)

Il apparaît une survie deux fois plus importante pour le groupe des malades ayant subi une irradiation lymphatique supérieure à 5 000 rad. Il faut noter que la proportion d'adénopathies avec capsule rompue (C+) est identique dans chaque groupe.

rents sont brisés sur des données thérapeutiques. Il convient de les reprendre en fonction de l'évolution clinique et thérapeutique.

Deux groupes sont à envisager : les N0 d'une part, les Np d'autre part (c'est à dire les ganglions palpables (p) rassemblant les N1, N2 et N3).

Sur une durée d'observation de 3 ans, la moitié des N0 sont devenus Np 67/134.

Les Np se répartissent donc en 111 malades avec adenopathie d'emblée (Np1) et 67 malades avec adenopathie secondaire (Np2), soit, au total, 178 malades $178/245 = 73\%$.

Parmi les malades avec adenopathie d'emblée (Np1), 83/111 ont subi un curage ganglionnaire, soit 75 %, parmi les malades avec adenopathie secondaire (Np2), 46/67 ont subi un curage ganglionnaire, soit 68 %. Les indications chirurgicales ont donc été réalisées dans une proportion équivalente pour les Np1 et les Np2.

La survie à 3 ans pour ces différentes catégories s'établit de la façon suivante :

N0 restes N0	48/67	72 %
Np1 opérés	39/83	47 %
Np2 opérés	18/46	39 %
Np1 non opérés	5/28	17 %
Np2 non opérés	2/21	10 %

On constate que la survie à 3 ans est très élevée pour les malades restés sans adenopathie (treize T1, douze T2a, dix huit T2b, quatorze T3a, dix T3b), elle est proche de la moyenne générale pour les Np opérés avec un pronostic un peu plus sévère pour les Np2 (cinq T1, onze T2a, quinze T2b, neuf T3a, six T3b) que pour les Np1 (un T1, dix huit T2a, vingt trois T2b, vingt deux T3a, dix neuf T3b), elle est très faible pour les Np non opérés, avec un pronostic lui aussi plus sévère pour les Np2 (nul T1, sept T2a, cinq T2b, quatre T3a, cinq T3b) que pour les Np1 (un T1, trois T2a, sept T2b, sept T3a, dix T3b). On constate en outre, que la proportion des adenopathies (Np1 et Np2) est identique pour les T2 et les T3 (89/119 T2 et 82/106 T3).

Parmi les 129 malades ayant subi un curage ganglionnaire (83 Np1 + 46 Np2), 90 présentent à l'examen histologique une ou plusieurs métastases ganglionnaires (N+ curage positif), 39 ne présentent que des ganglions sains (N- curage négatif). La voici la survie à 3 ans, sur un tableau plus détaillé :

	N +		N -		Total	
Np1	16/54	(29 %)	23/29	(79 %)	39/83	(47 %)
Np2	12/36	(33 %)	6/10	(60 %)	18/46	(39 %)
Total	28/90	(31 %)	29/39	(74 %)	57/129	(44 %)



Fig 3 Schema de dosimetrie etabli par la technique sur points Plan transversal Dose de base 59 et 58 rad/heure pour 1 mCi/cm Dose de reference (isodose en pointille) 50 rad/heure

de survie a 3 ans entre malades (T2 et T3) traites par radium ou indium 192 (respectivement 40 % et 50 %) a ete etablie des notre premier controle de denombrement avec 50 dossiers cette difference n a pas varie (a l unite pres) lors des denombrements ulterieurs (100 150 200 et finalement 245 dossiers) Cela prouve selon nous la possibilite de tirer des conclusions comparatives valables sur de petits nombres dans le cadre tres particulier des carcinomes epidermoides de la cavite buccale

La morphologie histo-pathologique de nos 245 cas est remarquablement homogene 93 % de nos cas sont des carcinomes differencies contre seulement 7 % de formes peu ou pas differencies (18 cas faiblement differencies trois T1 huit T2 sept T3)

La faible proportion de recidives au niveau de la tumeur primitive parmi les cas traites par indium 192 est manifestement en relation avec les progres techniques de ces epingles (technique par gouttieres vectrices) ou fils (technique par tubes plastiques) radio actifs Ces progres sont lies a trois facteurs principaux (1) la preparation non radio active qui permet d obtenir une mise en place rigoureuse et controlee du dispositif radifere (2) la continuite en toutes mesures des lignes radio actives qui permet d envelopper largement le volume cible a l interieur d une isodose de reference couvrant regulierement la surface muqueuse et les plans profonds du muscle lingual (figures 2 3 4) (3) notre systeme relationnel de dosimetrie avec son controle par la technique sur points qui permet de deceler avec precision d eventuels points

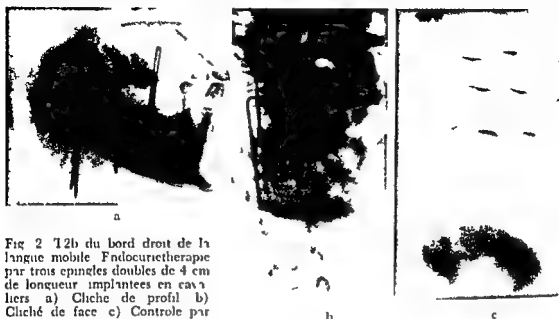


Fig 2 T2b du bord droit de la langue mobile. Endocurietherapie par trois epingles doubles de 4 cm de longueur implantees en cavariers a) Cliche de profil b) Cliché de face c) Controle par tomographie transversale

Discussion

Nous avons inclu dans cette même étude les epitheliomes de la langue mobile et ceux du plancher parce qu'il s'agit de la même famille tumorale. La plupart de ces carcinomes epidermoides naissent autour du sillon pchilingual qu'il s'agisse du bord de langue ou du plancher. Dans la mesure où l'on peut distinguer un epithelioma du plancher de celui de la langue mobile, nos contrôles de dénombrement ne nous ont montré aucune divergence tant au niveau de la clinique que du traitement et des résultats.

Nous avons éliminé les T4 de notre travail (tumeurs de plus de 5 cm de longueur et de plus de 3 cm d'infiltration), d'une part parce que notre protocole thérapeutique est différent et moins systématique (l'endocurietherapie n'a été appliquée que de façon occasionnelle) au niveau de ces grosses lésions, d'autre part parce que la plupart des T4 débordent largement les limites de la langue mobile et du plancher et ne peuvent plus être regroupés dans le cadre exclusif de ces structures.

Le caractère rétrospectif de notre étude d'une part, la subdivision de nos différentes catégories cliniques en de petits groupes cliniques d'autre part, ne nous ont pas permis de tirer de nos résultats des conclusions statistiquement rigoureuses. Il n'en reste pas moins que l'homogénéité clinique et biologique des malades atteints de carcinomes epidermoides de la langue mobile et du plancher permet, selon nous, de tirer des conclusions significatives à partir de faibles différences entre de petits groupes. C'est ainsi que la différence de 10 %



Fig 3 Schema de dosimetrie etabli par la technique sur points Plan transversal Dose de base 59 et 58 rad/heure pour 1 mCi/cm Dose de reference (isodose en pointille) 50 rad/heure

de survie a 3 ans entre malades (T2 et T3) traites par radium ou iridium 192 (respectivement 40 % et 50 %) a ete etablie des notre premier controle de denombrement avec 50 dossiers cette difference n'a pas varie (a l' unite pres) lors des denombrements ulterieurs (100 150 200 et finalement 245 dossiers) Cela prouve selon nous la possibilite de tirer des conclusions comparatives valables sur de petits nombres dans le cadre tres particulier des carcinomes epidermoides de la cavitte buccale

La morphologie histopathologique de nos 245 cas est remarquablement homogene 93 % de nos cas sont des carcinomes differencies contre seulement 7 % de formes peu ou pas differencies (18 cas faiblement differencies trois T1 huit T2 sept T3)

La faible proportion de recidives au niveau de la tumeur primitive parmi les cas traites par iridium 192 est manifestement en relation avec les progres techniques de ces epingles (technique par gouttieres vectrices) ou fils (technique par tubes plastiques) radio actifs Ces progres sont lies a trois facteurs principaux (1) la preparation non radio active qui permet d'obtenir une mise en place rigoureuse et controlee du dispositif radifere (2) la continuite en toutes mesures des lignes radio actives qui permet d'envelopper largement le volume cible a l'interieur d'une isodose de reference couvrant regulierement la surface muqueuse et les plans profonds du muscle lingual (figures 2 3 4) (3) notre systeme relationnel de dosimetrie avec son controle par la technique sur points qui permet de deceler avec precision d'eventuels points

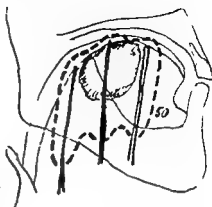


Fig. 4. Schéma de dosimétrie en vue de profil. On retrouve l'isodose de référence en pointe de 50 rad/heure.

froids ou points chauds et de les corriger par des variations dans la durée d'irradiation de telle ou telle ligne radio active.

Le pronostic de ces récurrences au niveau de la tumeur primitive reste cependant relativement favorable. C'est ainsi que sur 30 récurrences (radium ou iridium) qui ont pu être reprises par un traitement à visée curatrice, on constate 13 ans treize guérisons locales stables (sept guérisons obtenues par une nouvelle application d'endocurietherapie, six guérisons obtenues par une exérèse chirurgicale).

Nous constatons 85 cas de nécroses (dont 46 cas n'intéressant que les tissus mous et 35 cas intéressant l'os mandibulaire associé ou non aux tissus mous) parmi les 234 cas traités par endocurietherapie, soit 36 % de nécroses. La plupart de ces nécroses surviennent entre 3 et 12 mois après l'endocurietherapie. Dans 28 cas (soit 33 % du total des nécroses) une hospitalisation a été nécessaire du fait de l'infection, des douleurs et, par voie de conséquence, de la dénutrition.

Dans les 57 autres cas (67 % du total des nécroses), il ne s'agit que de radio lésions limitées et regressives sans retentissement général ou fonctionnel important.

La plupart de ces nécroses sont restées isolées : nous ne constatons que 10 cas associant nécrose et récurrence (quatre T2 et six T3). Aucun cas de décès n'a pu être imputé à la seule évolution de la nécrose. Deux faits intéressants sont à souligner.

1. La relation entre le nombre progressif de lignes radio-actives implantées (c'est à dire l'importance du volume traité) et la fréquence des nécroses.

	1-2	3-4	5-6	7-8	9-10
Nécroses	9 %	13 %	26 %	41 %	56 %

Cette relation explique, en partie, la plus grande fréquence des nécroses parmi les cas traités par iridium 192 (49/95 avec iridium 192 contre 36/139 avec radium 226).

2 L'absence de relation entre la durée de l'irradiation et la fréquence des nécroses (pour une même dose tumorale et dans les limites de nos étallements) entre 3 et 9 jours de durée d'irradiation la fréquence des nécroses reste la même.

Le pronostic de ces nécroses reste finalement favorable, nous constatons parmi les T2 et les T3 traités par endocurietherapie 60 % de survie à 3 ans pour les cas avec nécrose contre 34 % pour les cas sans nécrose.

L'appréciation clinique de l'envahissement métastatique des ganglions reste très incertaine. Ceci est particulièrement vrai pour les épithéliomas de la cavité buccale où l'on constate dans la grande majorité des cas des ganglions palpables dans la région sous-maxillaire sans qu'il soit possible — quelle que soit leur taille — d'affirmer s'il s'agit de ganglions inflammatoires de sous-maxillites ou de métastases. Dans 30 % des cas rappelons-le, qu'il s'agisse de Np1 ou de Np2 l'histologie après curage chirurgical s'est révélée négative (N—). Cette discordance fréquente entre la clinique et l'histologie prouve le caractère hasardeux de notre appréciation clinique. La question se pose donc de savoir s'il faut ou non recommander un curage chirurgical systématique des aires ganglionnaires satellites qu'il y ait ou non des ganglions cliniquement suspects. L'identité du pronostic des Np1 et des Np2 histologiquement N+ ne permet pas dans les limites de notre travail de répondre à cette question, seul un essai thérapeutique statistiquement contrôlé, permettrait d'y répondre (cet essai est actuellement en cours d'étude à l'Institut Gustave Roussy).

Parmi les onze malades qui n'ont pas subi d'endocurietherapie un seul a été traité par électro-chirurgie, il s'agissait d'un T1 N0 actuellement guéri à plus de 9 ans. Les dix autres (deux T2b, deux T3a et six T3b) ont tous présenté une poussée évolutive avant la date fixée pour l'endocurietherapie et n'ont été traités que par téléradiotherapie. À noter que l'un d'entre eux (T3b N0) est guéri depuis plus de 9 ans après 5 000 rad (télécobalt), tous les autres sont décédés en quelques mois.

Il reste à discuter les causes de décès à 3 ans.

Dix malades sont morts sans que nous ayons pu obtenir de précision sur la cause de leur décès.

Quarante-sept malades sont morts avec une récurrence au niveau de la tumeur primitive, dont vingt-huit avec une récurrence ganglionnaire associée, cinq avec des lésions à distance associées (métastases, autres cancers ou maladies intercurrentes), quatorze avec une récurrence linguale ou pélaennne isolée. Parmi ces quatorze cas on dénombre un T2a (traité par radium), cinq T2b (tous traités par radium), deux T3a (dont un traité par radium, l'autre traité par iridium), six T3b (dont deux traités par radium et quatre traités par iridium).

Soixante-dix malades sont morts avec une récurrence ganglionnaire cervicale,

dont vingt huit, déjà cités, avec une récurrence de la tumeur primitive associée, dix sept avec des lésions à distance associées, vingt cinq avec une récurrence ganglionnaire isolée. Les adénopathies sont une des causes principales de décès.

Vingt cinq malades sont morts avec des métastases, dont quatorze avec une récurrence buccale ou ganglionnaire (deux buccales, douze ganglionnaires), onze avec des métastases isolées ou associées à d'autres lésions à distance. À noter la prédominance des métastases médiastinales ou pulmonaires (quinze cas).

Quinze malades sont morts avec d'autres cancers, dont quatre avec une récurrence buccale ou ganglionnaire et onze isolées ou associées à d'autres lésions à distance. Tous ces autres cancers se situent au niveau des voies aérodigestives supérieures (quatre oro-pharynx, quatre hypopharynx, quatre œsophages, deux bronches), sauf un cas (rectum).

Vingt trois malades sont morts d'une affection intercurrente, dont cinq avec une récurrence buccale ou ganglionnaire, et dix huit isolées ou associées à d'autres lésions à distance. La plupart de ces affections intercurrentes étaient liées à des complications d'un ethylisme chronique.

Au total, les causes de décès sont largement déterminées par l'évolution de lésions à distance, associées ou non à des récurrences buccales ou ganglionnaires. Si l'on met à part les cas avec métastases associées à des récurrences buccales ou ganglionnaires (dont l'apparition est peut-être liée à la non stérilisation du processus loco-régional), on totalise finalement quarante cinq cas où le décès est inexorablement lié au développement de lésions à distance. On peut donc constater que 34 % des décès sont en relation inexorable avec des lésions à distance, 18 % de l'ensemble de nos malades meurent donc, avant 3 ans, de causes indépendantes de la seule évolution de la tumeur loco-régionale (tumeur primitive ou adénopathies cervicales).

Nous n'ouvrons pas de discussion avec les autres travaux sur le traitement des cancers de la langue mobile ou du plancher buccal pour la raison fondamentale qu'aucune comparaison significative n'est possible entre des travaux où la classification TNM n'a pu être préalablement établie en commun : autant d'auteurs indépendants, autant de classifications différentes (FLETCHER et coll 1962, SAXENA et coll 1967).

Conclusion

Parmi 245 malades atteints de carcinomes épidermoïdes de la langue mobile ou du plancher buccal (vingt T1, cent dix neuf T2, et cent six T3), la comparaison entre les cas traités par endocurthérapie avec radium (dosimétrie empirique) et ceux traités par iodium 192 en préparation non radioactive

(dosimetrie controlee) fait apparaitre une considerable difference dans la frequence des recidives (ou non sterilisations) au niveau de la tumeur primitive pour les tumeurs de moins de 4 cm de grand diametre sur un delai d'observations de 3 ans la frequence des recidives est de l'ordre de 3 % avec l'iridium 192, alors qu'elle atteint 35 % avec le radium. Les nouvelles techniques d'endocurietherapie avec preparation non radio-active ont donc reduit les risques de recidive locale a un taux presque negligeable.

Le pronostic reste cependant lourdement greve par la frequence des adenopathies cervicales : soixante dix de nos malades sont morts avec une recidive ganglionnaire (53 % des deces). Notre protocole de curage chirurgical et de teleradiotherapie postoperatoire a la demande clinique des adenopathies ne montre pas de difference significative dans la survie des malades avec adenopathie primitive ou adenopathie secondaire. Les formes histologiques d'adenopathies avec rupture capsulaire ont un pronostic particulierement severe.

Le pronostic est egalement assombri par la frequence des lesions a distance : metastases sans recidive loco-regionale, autres cancers, maladies intercurrentes. Quarante cinq de nos malades sont morts avec des lesions a distance (34 % des deces).

Au total la survie a 3 ans de nos 245 malades est de 46 %.

RÉSUMÉ

Parmi 245 malades atteints de carcinomes epidermoides de la langue mobile ou du plancher buccal la comparaison entre les cas traites par endocurietherapie avec radium et ceux traites par iridium 192 en preparation non radio active fait apparaitre une considerable difference dans la frequence des recidives au niveau de la tumeur primitive pour les tumeurs de moins de 4 cm de grand diametre sur un delai d'observation de 3 ans la frequence des recidives est de l'ordre de 3 % avec l'iridium 192 alors qu'elle atteint 35 % avec le radium. Les nouvelles techniques d'endocurietherapie avec preparation non radio active ont donc reduit les risques de recidive locale a un taux presque negligeable. Au total la survie a 3 ans de nos 245 malades est de 46 %.

SUMMARY

The results of the treatment of 245 patients with epidermoid carcinoma of the tongue or floor of the mouth by means of endocurietherapy with radium or iridium 192 a non radio active preparation are discussed. A considerable difference in the reaction of the primary growth over a three year period was evident. With tumours less than 4 cm in diameter the rate of recurrence was of the order of 3 per cent with iridium 192 while it reached 35 per cent with radium. These new techniques with non radioactive preparations thus reduce the risk of local recurrence to an almost negligible figure. The three year survival rate for the whole material was 46 per cent.

ZUSAMMENFASSUNG

Die Ergebnisse der Behandlung von 245 Patienten mit einem epidermoiden Carcinom der Zunge oder des Mundgrundes unter Anwendung der Endocurietherapie mit Radium oder Iridium 192 einem nicht radioaktiven Präparat werden besprochen. Im bedeutender Unterschied in der Reaktion des primären Wachstums während einer Drei-Jahres-Periode war deutlich. Bei Tumoren mit weniger als 4 cm im Durchmesser war die Rezidivfrequenz bei Iridium 192 in der Grossenordnung von 3 % während sie bei Radium 35 % erreichte. Diese neue Technik mit einem nicht radioaktiven Präparat vermindert somit das Risiko eines Rezidivs zu einer nahezu zu vernachlässigenden Grösse. Die Drei-Jahres-Überlebensrate für das gesamte Material betrug 46 %.

BIBLIOGRAPHIE

- BAILLET F. Etude de 280 épithéliomas de la langue mobile et du plancher de la bouche traités à l'Institut Curie Roussy de Janvier 1960 à Décembre 1965. Mémoire pour le certificat d'Études Spéciales d'Electro Radiologie. Paris 1968.
- FLETCHER G. H. and STOVALL M. Study of explicit distribution of radiation in interstitial implantations: correlation with clinical results in squamous cell carcinomas of anterior two thirds of tongue and floor of mouth. *Radiology* 78 (1962) 766.
- IFFERQUIN B. Précis de curiethérapie, p. 183. Masson et Cie Paris 1961.
- La brachycuriethérapie par iridium 192 dans les carcinomes épidermoïdes. O. R. L. In: Corso superiore di aggiornamento in oncologia clinica, p. 275. Ambrosiana Milano 1968.
- and LAYOS J. V. Dosimetry by tomography in interstitial curietherapy: point technique. *Amer. J. Roentgenol.* 87 (1962) 585.
- SAXENA V. S. and ALLT W. T. Cancer of the tongue. *J. Clin. Ass. Radiol.* 18 (1967) 274.
- VIGNIER H. Curie puncture du cancer du plancher de la bouche. Mémoire pour le certificat d'Études Spéciales d'Electro Radiologie. Paris 1961.

PORTABLE ^{90}Sr ^{90}Y APPLICATOR WITH INTERCHANGEABLE PLASTIC SHUNT FOR EXTRACORPOREAL BLOOD IRRADIATION

by

H. SKOLDBORN, H. ROSENGREN, INGER RAGNHULT and R. KELLGREN

Extracorporeal irradiation of blood has been used in the treatment of leukaemia (Lajtha et coll 1968 THOMAS et coll 1965) and for immunosuppression in conjunction with renal transplantations (HUME et coll 1966 MAGINN & BULLINORE 1968 ROSENGREN et coll 1968 PERS ON et coll 1969).

Repeat extracorporeal blood irradiation requires an arteriovenous shunt as described by SCRIBNER et coll (1960). The irradiation units usually utilize gamma rays from ^{60}Co or ^{137}Cs or beta rays from ^{90}Sr ^{90}Y (KUHLE et coll 1964 CROOKITE 1967 GILBERT & LAJTHA 1966 LAJTHA et coll 1968). The use of roentgen rays and ultraviolet light has also been reported (VALGIER 1969).

A plastic or silicone rubber tube coiled around a specially designed radiation source is usually employed for conducting the blood. A conventional cobalt machine may also be used, however, in which event the highest efficiency will be attained when the coil is suitably shaped and introduced into the collimator system close to the radiation source. ROSENGREN & SKOLDBORN (1968) applied the latter principle to a Siemens Gammatron III (3 000 Ci ^{60}Co).

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ZUSAMMENFASSUNG

Die Ergebnisse der Behandlung von 245 Patienten mit einem epidermoiden Carcinom der Zunge oder des Mundgrundes unter Anwendung der Endocurietherapie mit Radium oder Iridium 192 einem nicht radioaktiven Präparat werden besprochen. Ein bedeutender Unterschied in der Reaktion des primären Wachstums während einer Drei-Jahres-Periode war deutlich. Bei Tumoren mit weniger als 4 cm im Durchmesser war die Rezidivfrequenz bei Iridium 192 in der Größenordnung von 3 % während sie bei Radium 35 % erreichte. Diese neue Technik mit einem nicht radioaktiven Präparat vermindert somit das Risiko eines Rezidivs zu einer nahezu zu vernachlässigenden Größe. Die Drei-Jahres-Überlebensrate für das gesamte Material betrug 46 %.

BIBLIOGRAPHIE

- BAILLET F. Etude de 280 épithéliomas de la langue mobile et du plancher de la bouche traités à l'Institut Gustave Roussy de Janvier 1960 à Décembre 1965. Mémoire pour le certificat d'Études Spéciales d'Electro-Radiologie Paris 1968.
- FLETCHER G. H. and STOVALL M. Study of explicit distribution of radiation in interstitial implantations: correlation with clinical results in squamous cell carcinomas of anterior two thirds of tongue and floor of mouth. *Radiology* 78 (1962) 766.
- PIERQUIN B. Précis de curiethérapie p. 183. Masson et Cie Paris 1964.
- La brachycuriethérapie par iridium 192 dans les carcinomes épidermoïdes O. R. L. In: *Corso superiore di aggiornamento in oncologia clinica* p. 275. Ambrosiana Milano 1968.
- and GASOS J. V. Dosimetry by tomography in interstitial curiethérapie: point technique. *Amer. J. Roentgenol.* 87 (1962) 585.
- SARANA V. S. and ALLT W. T. Cancer of the tongue. *J. Canad. Ass. Radiol.* 18 (1967) 274.
- VICNIER H. Curiepuncture du cancer du plancher de la bouche. Mémoire pour le certificat d'Études Spéciales d'Electro-Radiologie Paris 1961.

total dose may be exceeded too soon. The alternative is fractionated irradiation with higher transit doses.

A light irradiation unit that the patient can carry on his person day and night has many advantages. The supply tubes can be made short and complete heparinization is probably unnecessary in many cases. Such an applicator has been described by HUME *et coll.* (1966) and by SUNDBOM *et coll.* (1969), although the average dose rate to the blood was far below the optimum.

The aim with the design to be described was to produce the lightest possible unit portable on the patient's upper arm and delivering 2 000 to 4 000 rad to 5 litres of blood per 24 hours. The bremsstrahlung dose rate to the most exposed point should not exceed 400 rad per week (at 2 000 rad/24 h to the blood). The irradiation element was to be a readily interchangeable sterilizable plastic shunt capable of withstanding at least a 24 hour continuous use. It should be possible to insert and withdraw the shunt without risk and there should be no hot spots when the unit is closed.

Design and operation data

Radiation source and shielding. The prototype in question was developed step by step in close collaboration with the Radiochemical Centre, Amersham, Buckinghamshire, England. The optimum combination of materials in a suitable radiation shield would probably have been beryllium/uranium (depleted ^{235}U). However, graphite was substituted for beryllium; the dimensions determined being 5 mm graphite nearest the source, followed by 2.3 mm uranium. The radiation source consists of ^{90}Sr ^{90}Y titanate incorporated in four aluminium foils, two on each side of the plastic shunt. The active area of each foil is 100 mm \times 8.5 mm and the total activity 3.3 Ci. A thin layer of stainless steel is interposed between the source and the plastic shunt. A 2.5 mm thick aluminium frame used in a previous model was kept in the present model. Hence the latter is less than optimal as to low weight and low bremsstrahlung with the 5 mm graphite plus 2.3 mm uranium specified.

The design is illustrated in Figs 1 and 2. The unit is housed in a 3.2 mm perspex casing, screwed together and open at one end. Outside this is a 0.5 mm stainless steel case fitted with an adapter of the same material, having a wall thickness of 1.25 mm (see Fig. 1). The latter makes insertion or removal of the plastic shunt a rapid and simple operation and at the same time serves as an adequate radiation shield in this direction. The total weight, including casing and stainless steel adapter, is 1.35 kg.

Plastic shunt. The plastic shunt is depicted in Fig. 2. It is moulded of rigid PVC in a series of 1 000 (Kautex Werke, Hangelar, Siegburg, West

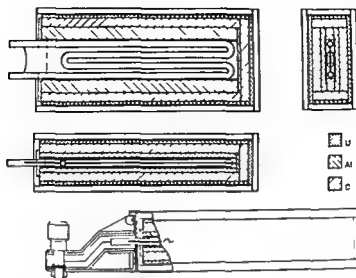


Fig. 1. Irradiation unit with plastic shunt in three projections and the assembled applicator with adapter.

The requirement for effective radiation shielding makes units employing ^{60}Co or ^{137}Cs sources heavy and bulky, and, moreover, necessitate fairly long tubes. Their greatest advantages lie in that they make possible high and variable dose rates and transit doses. The specially adapted Siemens Gammatron III enabled an average dose rate (to 4 litres of blood) of 1 300 rad/h and a transit dose of about 600 rad.

Irradiation units with ^{90}Sr ^{90}Y can be made lighter than those with ^{60}Co or ^{137}Cs but here too the hard bremsstrahlung makes effective shielding necessary. The equipment used by GILBERT & LAJTHA has provisions for a variable as well as a high dose rate. It requires, however, rather long tubes and complete heparinization of the patient and must therefore always be operated by skilled staff.

A comparatively light irradiation unit with 2.1 Ci ^{90}Sr ^{90}Y (weight including shielding 22 kg) is in use at Södersjukhuset (ROSENGREN et al. 1968). However, providing merely an average dose rate (to 4 litres of blood) of 100 rad/h and a maximal transit dose of 50 rad, it has primarily been used when relatively small doses have been required, e.g. in the treatment of lymphatic leukaemia.

Owing to the interchange of lymphocytes between the circulating blood and lymphatic tissue the blood must be irradiated continuously for a long time if a high proportion of the lymphocytes are to be affected by the irradiation. Hence a comparatively low transit dose may be preferable, or the admissible



Fig 3 The applicator in clinical use

The shunts are first carefully inspected and all with visible defects (including external width under 4.0 mm) are discarded. A series of 100 were thus approved and further investigated. Their mean weight was 5.23 g with a maximum deviation of 2% the maximum deviation in eighty of them not exceeding 1%. By weighing the contained water it was found that the average volume (including the non irradiated parts of the shunt about 13% of the total volume) was 3.49 cm³. Eighty eight of them deviated from the mean by at most 5% and were approved for clinical use. Their standard deviation was 2.1%. The deviation in weight and volume were uncorrelated.

A change in weight of 2% corresponds to a wall thickness change (provided the external measurements are equal) of about 0.01 mm. This alters the dose rate to the liquid in the shunt by at the most 2%. The dosage error is thus mainly a function of volume deviations.

If the plastic shunts are selected and approved for clinical use in accordance with these premises the average dose rate to the blood will be reproducible within $\pm 10\%$ an acceptable value for the clinical applications intended. A more comprehensive series of measurements by Fricke dosimetry will nevertheless be carried out in order to check the conclusions reached.

Operation The plastic shunt is sterilized with fluid just like a plastic catheter. It is coupled to the Scribner shunt by two silicone rubber tubes.



Fig. 2. The applicator and a plastic shunt.

Germany). The channel in the irradiated portion of the shunt (between the active foils) is approximately 400 mm long and its volume is 3 cm³. The wall thickness is 0.45 to 0.50 mm and its external width 4 mm. The cross section is 'oval' with somewhat flattened margins nearest to the active foils. It proved impossible to mould a rigid PVC shunt with a rectangular cross section.

Average dose rate and transit dose. Two methods were used for measurement of the average dose rate to the circulating blood. At the first measurement, which was made at Amersham, 40 lithium fluoride dosimeters were uniformly distributed in the irradiated portion of the plastic shunt. In the second series of measurements a solution of ferrous sulphate was used as dosimeter. A volume of 250 ml of this Fricke solution was pumped through the plastic shunt via the same system of silicone rubber tubes as in clinical practice. After correction for oxidation by the tubes and shunt the average dose to the solution could be estimated. Repeat measurements with seven shunts yielded a mean dose of 4.25 krad to 5 litres of blood in 24 hours with a maximum deviation of 6.2%. The adopted G value was 15.4 (TREGGIE 1967).

The value of 4.25 krad/24 h to 5 litres of blood corresponds to an average dose rate in the irradiated part of the shunt (about 3 cm³) of some 2.9×10^5 rad/h or 4.9 krad/min. The properties of PVC material deteriorate by some 25% after irradiation with 1.1×10^6 rad, according to COLLINS & CALGINS (APEX 261 1956). Hence without material damage due to the irradiation, the plastic shunt may be used without risk for at least two weeks continuously, in other words longer than it would ever be used in clinical practice (15 days corresponds to 63 krad to 5 litres of blood).

The flow velocity in the shunt in clinical practice was determined to about 180 cm³ per minute, corresponding to a transit dose of 80 rad.

Accuracy. If the patient's blood volume is known, the accuracy of the dosage will depend mainly on the properties of the plastic shunt (variations in wall thickness and volume).



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Operation The plastic shunt is sterilized with fluid just like a plastic catheter. It is coupled to the Scribner shunt by two silicone rubber tubes so

that the applicator can be attached to the upper arm. The shunt is then introduced into the irradiation unit, a rapid procedure entailing no risks whatsoever from radiation aspects if properly done. It is shown in Fig. 3 how the applicator is mounted on the patient's upper arm.

Radiation shielding. The absorbed surface dose rates at the centre of the top and bottom sides of the fully enclosed applicator were 3.5 rad/h. Accordingly, the dose to the most exposed point on the upper arm will not exceed 400 rad at an average dose of 20 000 rad to 5 litres of blood. This roughly agrees with the premises stated in the introduction. Obviously the dose to other parts of the body will be much lower. The following dose rates have been measured with lithium fluoride dosimeters: axilla 225, nape of neck 15, sternum 50 and groin 25 mrad/h.

The dose rate at a distance of 1 m from the top or bottom of the applicator is 5 mrad/h but less in other directions. Staff and others should thus not be unnecessarily in the patient's immediate vicinity. With this rule observed no problems should arise. Radiation doses to members of the staff are at present checked by film dosimeters.

Clinical use. The blood irradiation applicator was ready for clinical use in July 1969 and so far (October 1969) it has been used in only five patients for the purpose of immunosuppression prior to kidney transplantation. Owing to its interchangeable plastic shunt and convenient dimensions it is easy to apply and appears to cause a minimum of inconvenience to the patient.

We have up to the present been reluctant to waive heparinization as a protection against coagulation in the irradiation unit. Experience indicates that the most critical points are the attachments to artery and vein. Yet, irradiation without heparinization will be tried and we contemplate an average dose of at least 20 000, at the most about 30 000 rad, before renal transplantation is envisaged.

The brief observation period prevents any prediction concerning the long term immunosuppressive effects in these cases.

Development prospects. As mentioned before the applicator might be improved. A minimum of aluminium around the irradiation source would reduce the intensity of the bremsstrahlung. Any beta radiation not absorbed in the shunt with contained blood will then be largely stopped by graphite (atomic number 6) instead of, as now, by aluminium (atomic number 13) and graphite. Simultaneously the irradiation unit would become smaller and lighter with uranium plates of the same thickness. If beryllium (atomic number 4) were used in place of graphite there would be a further gain, although the applicator would then become much more expensive. If the radiation source could be made

linear with the same outline as the plastic shunt it would probably be feasible to increase the dosage yield per Ci ^{90}Sr ^{90}Y .

The effectiveness of the irradiation unit described is around 13 % i.e. the blood circulating in the shunt absorbs 13 % of the energy released at the source (26 % if only the radiation emitted into the solid angle 2π is considered).

The average dose rate to the blood may be affected significantly by changes either in the activity of the source or in the shunt volume. The shunt described was designed with great attention to the risk of blood coagulation. This hazard was deemed greater in a flat irradiation element which moreover would greatly affect the transit dose owing to differences in blood velocity through a rectangular cross section. Experiences with the applicator will act as a guide in the design of its successors.

SUMMARY

A portable ^{90}Sr ^{90}Y applicator for extracorporeal irradiation of blood providing an average dose rate of 4.3 krad/24 h per 5 litres is described. Accuracy of dosage and radiation hazards are discussed. It is likely that the applicator will be used for immunosuppression and in the treatment of leukaemias.

ZUSAMMENFASSUNG

Eine transportable Apparatur für extracorporale Bestrahlung des Blutes mittels ^{90}Sr ^{90}Y mit einer Intensität von 4,3 krad/24 Stunden pro 5 Liter wird beschrieben. Die Genauigkeit der Dosierung und die Bestrahlungsfahren werden diskutiert. Anwendungsmöglichkeiten für die Methode sind die Leukämien und die Unterdrückung der Autoimmunisationsvorgänge.

RÉSUMÉ

Description d'un applicateur portable de ^{90}Sr ^{90}Y pour l'irradiation extracorporelle du sang délivrant un débit de dose moyenne de 4.3 krad/24 heures et par 5 litres. Les auteurs étudient la précision, la dosimétrie et les dangers d'irradiation. Il est vraisemblable que cet applicateur sera utilisé pour l'immunosuppression et dans le traitement des leucoses.

REFERENCES

- COLLINS C. G. and CALVERT V. P. Radiation damage to elastomers, organic liquids and plastics. *APF* 761 (1956).
- CROWTHER E. P. Extracorporeal irradiation of the blood and lymph in the treatment of leukaemia and for immunosuppression. *Ann intern Med* 67 (1967) 415.
- FRECHET V. O. Calibration of the ferrous sulfate dosimeter by ionometric and calorimetric methods for radiations of a wide range of energy. *Radiat Res* 31 (1967) 256.

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The brief observation period prevents any prediction concerning the long term immunosuppressive effects in these cases.

Development prospects. As mentioned before the applicator might be improved. A minimum of aluminium around the irradiation source would reduce the intensity of the bremsstrahlung. Any beta radiation not absorbed in the shunt with contained blood will then be largely stopped by graphite (atomic number 6) instead of, as now, by aluminium (atomic number 13) and graphite. Simultaneously the irradiation unit would become smaller and lighter with uranium plates of the same thickness. If beryllium (atomic number 4) were used in place of graphite there would be a further gain, although the applicator would then become much more expensive. If the radiation source could be made

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EVALUATION OF REGIONAL LYMPH DRAINAGE FROM MAMMARY GLAND AND HAND TO AXILLARY NODES

by

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CH ÅKREN

The lymphatics of the breast used to be outlined anatomically by post mortem injection studies. Indirect evidence of the *direction* of the lymph flow was also obtained in examinations of the sites of metastases associated with carcinoma of the breast. In recent years more delicate techniques have been adopted to investigate the lymphatic drainage. Thus local injection of dyes and radioactive isotope tracers in the mammary gland have been used: these are transported to the regional nodes by the natural lymph flow (HULTBORN & JONSON 1955, HULTBORN et coll 1955, 1955 and TURNER WARWICK 1959). Furthermore direct lymphography from the dorsum of the hand has made it possible to demonstrate the lymphatics and lymph nodes in the axilla: this method has been evaluated in the diagnosis of lymph node metastase (HENDALL et coll 1963, HULTEN et coll 1966). The latter method has however been criticized because it cannot be taken for granted that the lymph flow from the arm and breast is diverted to the same group of axillary lymph nodes. This is of surgical interest.

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- GILBERT C W and LAJTHA I G A beta ray extracorporeal irradiator *Phys in Med Biol* 11 (1966) 281
- HUME D M LEE H M WILLIAMS G M et coll Comparative results of cadaver and related donor renal homografts in man and immunologic implications of the outcome of second and paired transplants *Ann Surg* 164 (1966) 352
- KUHL O A KIPFERGER W HUSZACH D W and CRONKITE F P Cobalt 60 irradiator for extracorporeal blood irradiation *Nucleonics* 22 (1964) 79
- LAJTHA L G CARRET J V TURNER L et coll Extracorporeal irradiation of the blood in leukemic patients *Brit J Haemat* 16 (1969) 39
- MACINN R R and BULLMORF J A Extracorporeal irradiation of the blood in renal homograft rejection *Brit J Radiol* 41 (1968) 127
- PERSSON B ROSENGREN B BERGENTZ S E and HOOD B Evaluation of preoperative extracorporeal irradiation of the blood in human renal transplantation *Transplantation* 7 (1969) 534
- ROSENGREN B och SKOLDBORN H Extrakorporal blodbestrålning (In Swedish) *Svenska Läk Tidn* 65 (1968) 2751
- BERGENTZ S E HOOD B and LINDAHL KIRSLING K Extracorporeal irradiation of blood A clinical study in candidates for renal transplantation *Scand J Urol Nephrol* 2 (1968) 58
- SCHIBNER R H CANER J E Z BULL R and QUINTON W The technique of continuous hemodialysis *Trans Amer Soc Artif Intern Organs* 6 (1960) 88
- SUNDBOM I BARRING N E HOLMER A och NILSSON B Barbra ⁹⁰Sr ⁹⁰Y bestrålningsenhet for extrakorporal bestrålning av blod (In Swedish) *Nord Med* 82 (1969) 1264
- THOMAS F D FISTEIN R B FISCHBACH J W et coll Treatment of leukemia by extracorporeal irradiation *New Engl J Med* 273 (1965) 6
- VAUGIER G Irradiation U V extracorporelle du sang Thèse Marseille 1969

(1966) Patent blue was injected subcutaneously and interdigitally leading to blue staining of the lymph vessels of the dorsum of the hand. One of the vessels was exposed and cannulated with a fine bore polythene catheter, the contrast medium being Lipiodol Ultra Fluide 38 %. An automatic injection pump was used for the injection at a rate of 0.05 to 0.1 ml/minute of 4 to 7 ml of the medium which was heated to around 50° C to reduce its viscosity.

One minute after the start of the injection, objective verification of the intra lymphatic injection was obtained by roentgen examination. The axillary and supraclavicular regions were examined immediately after the contrast medium injection. A further roentgen examination was made up to about 24 hours later, as the lymph nodes could be more readily examined when the lymph vessels had been emptied of their contrast medium content.

C Operative procedures Radical mastectomy was mostly performed the day after lymphography. In order to facilitate subsequent topographic orientation of the whole specimen, and localization of the nodes in the axillary fat, silver clips or steel wire sutures were affixed in positions corresponding to the axillary vein and the thoracic wall. The axilla was examined roentgenographically after the lymph node dissection and any lymph nodes containing Lipiodol that had been overlooked were identified and removed. As may be seen from Fig 2a the lymph nodes in the axilla contained Lipiodol before mastectomy while after mastectomy (Fig 2b) no contrast medium remained in the axilla but lay in the lymph nodes in the supraclavicular region which was not included in the operation field.

D Roentgen examination of the specimen Fine grain double coated double wrapped industrial film Kodak Crystallex was used. The breast, the axillary specimen and the separately removed fatty tissue and lymph nodes were placed on the film either directly or, to prevent wetting, protected by thin absorbent paper.

The roentgen source in all but one case was a Siemens Pantix tube (type P 125 30 50) the smaller focus measuring effectively 1.2 mm × 1.2 mm was employed. The exposure data were: focus-film distance 80 cm, 320 mA, 35 to 40 kV, exposure time 2.5 to 4 seconds. The specimens of one of the later cases were examined with the CGR Senographie apparatus made especially for soft tissue radiography and having an effective focus of 0.7 mm × 0.7 mm. The exposure data in this case were: focus-film distance 25 cm, 25 mA, 25 to 30 kV, exposure time 2 seconds.

The films were developed in a Pako XM film processor with Gevaert 137 developer for 3 minutes at 21° C.

The detail of a roentgenogram is affected by the radiographic contrast and

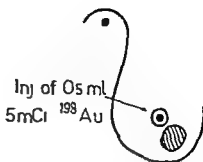


Fig. 1. Carcinoma of the lower medial quadrant of the breast. The arrow indicates the site of injection of radioactive gold cranio-lateral to the nipple. Palpable node in the axilla.

A method has been worked out for assessing the distribution of the lymph from the arm and the breast and to investigate whether the lymph drainage from these two areas is separate in the axilla. This includes injection of a tracer in the breast combined with lymphography from the dorsum of the hand and subsequent identification of the axillary lymph nodes in the operation specimen. A description of the method is given in the present paper, results and clinical implications will be discussed in forthcoming publications.

Investigation procedures

Patients with clinical roentgenologic and cytologic evidence of carcinoma of the breast but without clinical signs of metastases in the axilla were examined. The procedures were as follows: injection of a tracer substance (^{198}Au) into the breast, lymphography with Lipiodol, radical mastectomy and postoperative investigation of the specimen, including roentgen examination to demonstrate the Lipiodol, measurement of radioactivity, autoradiography and finally histologic examination.

There are two methods of demonstrating the Lipiodol: roentgen recording and histologic examination, and two indicators for radioactivity: direct measurement and autoradiography.

A. Tracer method (^{198}Au). The injections of a colloidal suspension of metallic ^{198}Au (GCS-1P and GCS-4P, the Radiochemical Centre, Amersham, England) were made with a fine bore needle into the parenchyma (Fig. 1). A volume of 0.5 to 1.5 ml and an activity of 1.5 to 5.0 mCi were used. The particle size ranged up to 200 Å (GCS-1P) in two cases and 200 to 300 Å (GCS-4P) in six cases. No external counting was made.

B. Lymphography. This was performed next day. The reason for this sequence was that Lipiodol may impede the introduction of ^{198}Au . The technique was that described by KIMMONT (1954), WALLACE et coll. (1961) and HULTÉN et coll.

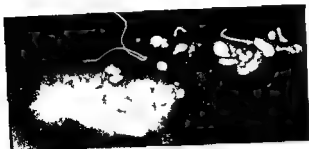


Fig 3 Specimen lymphadenogram

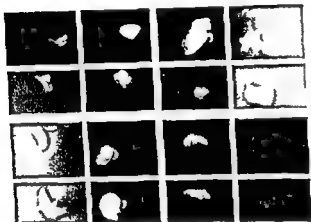


Fig 4 Sample lymphadenograms with a series of lymph nodes dissected out

residual lymph nodes were then dissected out. The original location of some of these nodes found at the second or third stage of dissection could not be estimated and these nodes were recorded as from an unknown region.

The breast and pectoral muscles were investigated and great care was taken in searching for the nodes dorsally on the major pectoral muscle including the interpectoral nodes.

The magnifying glass mentioned earlier was used in the dissection of the specimens.

An almost exact anatomic location of the major part of the lymph nodes in the axillary fat as well as of those removed separately was possible by detailed orientation during the operation. The individual nodes could be identified and transferred to an anatomical diagram and for practical purposes arranged in conventional groups.



Fig. 2 a) Lymphadenogram before radical mastectomy. Axillary and supraclavicular lymph nodes containing Lipiodol, a few lymph vessels and extravasated contrast medium in the axilla. b) Examination after radical mastectomy with all the axillary nodes containing Lipiodol removed but with supraclavicular nodes beyond the operation field.

definition, which in turn are governed by many factors the discussion of which is beyond the scope of this paper. The films obtained with the technique described generally had good detail and demonstrated clearly the lymphographic structure when examined through a lens (+5.75 diopters, focal length 17.4 cm, diameter 11.7 cm), even in nodes not larger than 1 mm. Small lymph nodes not carrying the contrast medium were also sharply outlined.

With guidance of the detailed roentgenogram (specimen lymphogram, Fig. 3) the lymph nodes were finally dissected out from the axillary fat. The individual lymph nodes were mapped out, numbered and arranged in series for individual roentgen examination, using the kind of industrial film referred to above (Fig. 4). The specimens, which were kept in formalin, should then be as dry as possible since the formalin after penetrating the envelope dissolves the coating of the film (Fig. 5).

A problem encountered during the roentgen examinations was that the intensity of the radiation emitted from the ^{198}Au in the lymph nodes sometimes blackened the film to such an extent that the images of the nodes were partly or even totally obscured (Fig. 6a). This could be avoided by increasing the specimen-film distance, thereby decreasing the radiation intensity in the film plane according to the inverse square law. A small increase of 15 mm was found to be enough and with a focus-film distance of 80 cm did not impair the definition of the roentgenogram to a noticeable degree (Fig. 6b, b' and c).

The remaining axillary fat was always re-examined roentgenologically and any

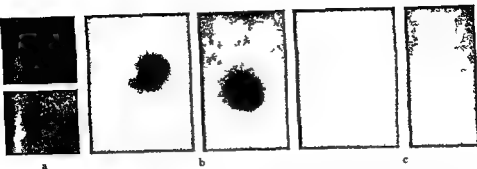


Fig 3 a) The lymph node in the lower image is small but easily identified by its Lipiodol content. Section in upper image had high radioactivity blackening the film and obscuring the node (see also fig 4). b) and c) Roentgenograms of small lymph nodes with respectively Lipiodol and high radioactivity. The specimens corresponding to (b) were placed directly on the film envelope while for those corresponding to (c) the distance film specimen was increased by 15 mm which was sufficient to prevent blackening.

G Histologic technique The dissected lymph nodes were fixed in 10% neutral formaldehyde solution for 12 to 18 hours and routinely prepared for paraffin embedding. The blocks were cut at 4μ and primarily two to four sections were obtained from each block and mounted on slides. This procedure always took place immediately after the operation to allow autoradiography within two and a half days after the dissection of the nodes. The sections used for autoradiography were then stained by the van Gieson method for histologic examination for signs of contrast medium deposition of ^{199}Au or existence of metastases. Further sections at deeper levels were taken from the paraffin blocks when the specimens proved radioactivity but no evidence of activity was found at autoradiography and no lymph nodes could be demonstrated in the stained sections.

Discussion

Demonstration of Lipiodol Only vacuoles or irregular empty cavities remained after the droplets or lakes of Lipiodol had been dissolved during the histotechnical procedure. Most nodes had contained large amounts of the medium to give the section a characteristic honeycomb structure (Figs 7 and 8). Even some destruction of the parenchyma had occurred in heavily loaded nodes.

Moderate amounts of the contrast medium when present were confined to the sinusoids of the node and could usually be easily identified. Difficulties can arise however in differentiating Lipiodol vacuoles from lobules of fat cells or single fat cells which are common as an atrophic phenomenon in axillary lymph nodes.



Fig. 5. Halo around a lymph node caused by the dissolving action of the formalin on the film.

When this part of the investigation was terminated, all lymph nodes and the axillary fat were kept for further quantitative measurements of radioactivity, autoradiography and histologic examination.

E. Quantitative measurements of radioactivity. After dissection of the specimens, each lymph node and the axillary fat were placed separately in a Picker Autowell II Sample Changer, having a well crystal, 3 inches in diameter. The lower level discriminator was set at 350 keV and the channel width was 130 keV. The count rates ranged from less than one hundred cpm to several hundred thousand cpm. All the samples were first measured for 6 seconds and specimens with low activity (less than 100 counts in 6 seconds) were then measured again for one minute. This method is most sensitive. High activity in the remaining axillary tissue indicated the probable presence of any overlooked lymph nodes, and such a specimen was redissected.

F. Autoradiography. Five to eight days after injection of the radioactive gold into the breast, autoradiographs of the histologic sections of the lymph nodes were made according to a simple contact method. The same sections were then stained and examined histologically. This technique was considered adequate for the purpose because of the high sensitivity of the quantitative measurements in the Picker Autowell II Sample Changer.

We used an ordinary roentgenographic cassette and Agfa Gevaert Curix RP film. When the cassette was closed, it pressed the film against the histologic sections. Two or three series of sections from different levels were selected, and the exposure times were from 24 hours to one week. A suitable level of activity is necessary for good results — if blackening of the film was too dense and unsharp the autoradiographic procedure was repeated after a few days, when the isotope had decayed (Fig. 7). With the use of this method it is of course important that the section in the paraffin block should really lie at the level of the lymph node. If this is not the case, one of the measurements of radioactivity would be lost — namely the autoradiographic one. This occurred in a few lymph nodes of very small size.



Fig 8 Part of lymph node with signs of Lipiodol storage. Numerous round ovoid vacuoles through the parenchyma. The larger droplets tend to coalesce into lakes of a regular shape. van Gieson $\times 30$.



Fig 9 Part of lymph node with marked fat in omentum (lower half) and a few Lipiodol vacuoles (+) between the fat cells. It may be noted that the fat cells are of polygonal shape and relatively uniform size. van Gieson $\times 10$.

oids and the difficulty lay in differentiating the vacuoles from artefacts. The latter could sometimes be correctly identified when they were connected with other artefacts such as rifts; furthermore they often had an irregular shape compared to the spherical Lipiodol droplets. The true nature was sometimes in doubt when the slides had been prepared and stained for the demonstration of carcinoma metastases.

The nodes in five cases were exposed to Lipiodol for only about 24 hours before fixation in formalin and in these cases there were no inflammatory reactions around the droplets of contrast medium except a few eosinophilic granulocytes in occasional nodes. If the experimental model allows exposure for e.g. 48 hours numerous leucocytes and with even longer exposure many foreign body giant

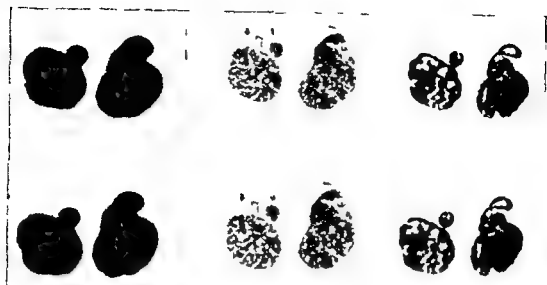


Fig 7 Autoradiograms exposed 12—15 July to the left and those exposed 15—16 July to the right in the centre the histologic sections

(Fig 8) The distinction may, however, with some experience generally be made. The Lipiodol vacuoles vary considerably in size and tend to coalesce into small lakes. The size of the fat cells is much more constant, and these cells often form small lobules protruding from the hilus of the node. Even if occasional fat cells may have the shape of empty spheroids, like the Lipiodol vacuoles, they usually are somewhat polygonal (Fig 9).

The cell membrane or traces of the membrane may often be identified although this may sometimes be difficult or even impossible. However, the employment of thicker sections of about 10 μ or more would much increase the likelihood of identifying the cell membrane or its residues in doubtful specimens. It would then be necessary to cut consecutive sections from the same block at 4 μ to investigate e.g. carcinoma metastases. The fat cell nucleus was usually hard to identify against the numerous parenchymal cell nuclei of the node. A thorough study of the sinusoids and the adjacent lymph vessels, where the Lipiodol vacuoles could be easily identified, was of great value, as was a comparison with the cells of the surrounding fat tissue. With these characteristics, signs of contrast storage, if present to a moderate degree, can always be demonstrated histologically in sections stained by van Gieson or hematoxylin eosin, i.e. methods suitable for the investigation of carcinoma metastases.

Considerable difficulties may arise in identifying the Lipiodol histologically, in nodes with very small amounts stored in droplets of about 10 to 15 μ and too small to be identified in the lymphograms. The medium was located in the sinus

mononuclear cells probably reticular cell or macrophages which have phagocytized the grains. The grains are homogenous and stain black in specimens prepared with van Gieson. Their chemical nature is not clear. These observations are in accordance with those of KAUTSON & NORIN (1956).

There seems to be a rough but not clear correlation between estimations of high activity and the amount of demonstrable grains. On the other hand occasional nodes of high activity have scant or no visible precipitations possibly due to dissolution during the histotechnical procedure as the substance is water soluble.

The rather time consuming method advanced by MELLGREN (1952), which includes vacuum drying and paraffin embedding in vacuo must be used to avoid dissolution and diffusion phenomena these however are of minor importance as stated by MELLGREN et coll (1954). The histologic demonstration of gold pigment in routine preparations may thus be convenient although extremely minute amounts may be overlooked.

A reduction of the number of lymphocytes appears to occur in nodes with high activity and areas rich in clusters of ^{199}Au particles even if no traces of lytic cells could be demonstrated (Fig 10). This observation is interesting as a true reduction of cells around the aggregates of ^{199}Au could mean increased cell deaths due to a local radiation effect from the precipitates. The local dose of radioactivity in one single lymph node may with the present technique correspond to as much as 1 500 rad per 24 hours and the exposure time multiplies this two or three times. As is known from other data, this is sufficient to cause cell depopulation in exposed lymph nodes (TROWELL 1952) an observation that requires investigation. One error would be any oedema following irradiation as this would influence to a certain degree the number of cells per unit area. No obvious signs of such oedema were seen in the nodes examined. The histologic changes observed in connection with the ^{199}Au aggregates were insignificant, and no indications that the existence of the tracer within the node in any way interfered with its capacity to take up the contrast medium were noted. This is also borne out by the fact that about 85 per cent of the nodes contained both ^{199}Au and contrast medium.

SUMMARY

A method for the evaluation of the distribution of the lymph drainage from different parts of the body to a particular lymph node region is presented. It consists of injection of a radioactive tracer in one part of the body and a lymphographic medium in another. The method may be of considerable value in clarifying certain unsolved clinical problems associated with surgery and radiotherapy of mammary carcinoma.

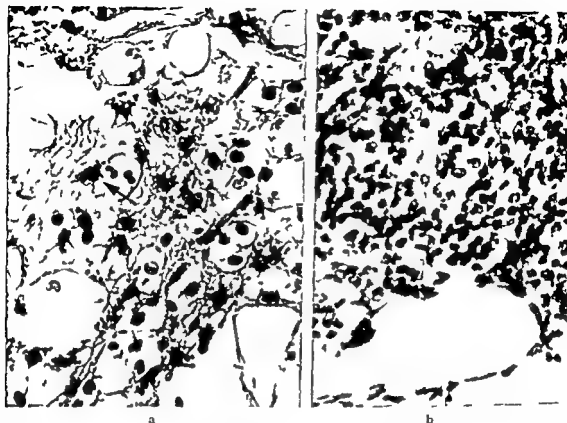


Fig 10 A part of a lymph node with numerous aggregates and grains of gold partly fixed to phagocytizing macrophages (arrow) is shown in (a) which contains few lymphocytes compared with a corresponding area of another lymph node (b) with no radioactivity and no visible gold van Gieson $\times 530$

cells, will surround the droplets (TJERNBERG 1962, ALFRETTE et coll 1964, HULTEN et coll 1966). The existence of such cells around spherical vacuoles clearly differentiated these as Lipiodol residues.

The use of a technique that includes fixation and paraffin embedding is necessary for the proper demonstration of carcinoma metastases. This puts a limit to the possibilities of staining the Lipiodol, as it is dissolved more or less totally during the procedure. Attempts to stain any residues of this contrast medium have so far failed.

Demonstration of ^{198}Au Clusters of ^{198}Au particles can be demonstrated on routine preparations stained with van Gieson's solution. The ^{198}Au forms either single ovoid grains of about $2\ \mu$, or irregular aggregates of 10 to $15\ \mu$. They are either diffusely powdered over the specimen or lie in considerable amounts in the sinusoids (Fig 10a), often along the stromal septa. They sometimes cover large

INTERCOMPARISONS OF ABSORBED DOSE IN COBALT 60 TELETHERAPY USING MAILED LiF DOSIMETERS

II Results for Canada and six Asian countries

by

PAUL M PFALZNER and S JAYARAMAN

The object and methods used in the postal dose intercomparisons instituted by the Dosimetry Section of the International Atomic Energy Agency have been described earlier together with results for the first intercomparison which comprised a group of 19 radiotherapy centres in six countries (PFALZNER & MALO ALVAREZ 1968). Since then the same procedures were made available to 14 Canadian participants (Table 1a) and 15 participants in six countries of Asia (Table 1b).

This report gives a brief description of the procedures (essentially those of the first intercomparison) and an analysis of the results for these two groups of participants.

Method At approximately monthly intervals each participant received from and returned to IAEA one set of LiF capsules up to a total of three sets. A set consisted of three test capsules and two control capsules each capsule containing about 150 mg of ConRad type 7 phosphor. Participants were instructed to irradiate the test capsules one at a time with a ^{60}Co beam of 10 cm \times 10 cm field size within a plastic water phantom (supplied to them by IAEA) which ensured that the capsule was held at 5 cm below the surface of the water. The

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ZUSAMMENFASSUNG

Eine Methode zur Abschätzung des Lymphabflusses von verschiedenen Körperregionen zu bestimmten Lymphdrüsengruppen wird vorgelegt. Sie besteht aus Einspritzung von einem radioaktiven Isotopen in einen Teil des Körpers und einem lymphographischen Mittel in einen anderen Teil. Die Methode ist von besonderem Wert zur Aufklärung klinischer Probleme in der Chirurgie und Strahlentherapie des Brustdrüsenkarzinoms.

RÉSUMÉ

Les auteurs présentent une méthode permettant d'établir la distribution du drainage lymphatique venant des différentes parties du corps et arrivant à un groupe donné de ganglions lymphatiques. Cette méthode consiste à injecter un traceur radioactif dans une partie du corps et à faire une lymphographie dans une autre partie. Elle peut avoir un intérêt considérable pour éclairer certains problèmes cliniques non résolus qui sont en relation avec le traitement chirurgical et radiothérapique du cancer du sein.

REFERENCES

- AVFRETTE H. F., HUDSON R. C. and FERGUSON J. H. Lymphangiadenography: Applications in the study and management of gynecologic cancer. *Cancer* 17 (1964), 1093.
- HULTBORN A. and JOHANSSON L. I. The use of colloidal Au^{198} for the detection of lymph nodes in radical excision of the breast. *Acta radiol.* 43 (1955), 132.
- LARSSON L. G. and RAGNULT I. The lymph drainage from the breast to the axillary and parasternal lymph nodes studied with the aid of colloidal Au^{198} . *Acta radiol.* 43 (1955), 52.
- — A study of the lymph drainage of the lower limb with the use of colloidal radiogold (Au^{198}). *Acta radiol.* 43 (1955), 139.
- HULTEN L., ÅHREN Ch. and ROSENCRANTZ M. Lymphangiadenography in carcinoma of the breast. Comparative clinical roentgen and histologic appraisal of the method for the demonstration of lymph node metastases. *Acta chir. scand.* 132 (1966), 261.
- KENDALL H. E., ARTHUR J. F. and PATFY D. H. Lymphangiography in carcinoma of the breast. A comparison of clinical, radiological and pathological findings in axillary lymph nodes. *Cancer* 16 (1963), 1233.
- KINMONT J. H. Lymphangiography in clinical surgery and particularly in the treatment of lymphoedema. *Ann. roy. Coll. Surg. Engl.* 15 (1954), 300.
- KNUTSON F. and NORIN T. Localization and effect of injected radioactive gold (Au^{198}) in the treatment of malignant tumours. II. *Acta path. microbiol. scand.* 38 (1956), 447.
- MILLGREN J. Histochemical preparation for autoradiography. *Exp. Cell Res.* 4 (1952), 689.
- KNUTSON F. and NORIN T. Localization and effect of injected radioactive gold (Au^{198}) in the treatment of malignant tumours. I. *Acta path. microbiol. scand.* 34 (1954), 393.
- TJERNBERG B. Lymphography. An animal study on the diagnosis of Vx2 carcinoma and inflammation. *Acta radiol.* (1962) Suppl. No. 214.
- TROWEL O. A. The sensitivity of lymphocytes to ionizing radiation. *J. Path. Bact.* 64 (1952), 687.
- TURNER WARWICK R. T. The lymphatics of the breast. *Brit. J. Surg.* 46 (1959), 574.
- WALLACE S., JACKSON L., SCHAFER H. et coll. Lymphangiograms. Their diagnostic and therapeutic potential. *Radiology* 76 (1961), 179.

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Table 1

Institutes participating in the intercomparison

Table 1a — Canada		Table 1b — Asia	
Department of Public Health Cancer Clinic	Edmonton Alta	Government Cancer Institute	Maharagama Ceylon
British Columbia Cancer Institute	Vancouver B. C.	Tata Memorial Hospital	Bombay India
Victoria Cancer Clinic	Victoria B. C.	Cancer Institute	Madras India
Saint John General Hospital	Saint John N. B.	National Institute of Radiological Sciences	Chiba Japan
Victoria General Hospital	Halifax N. S.	Kyushu University Hospital	Fukuoka Japan
Ontario Cancer Foundation Kingston Clinic	Kingston Ont.	Research Institute for Nuclear Medicine	Hiroshima Japan
Ontario Cancer Foundation London Clinic	London Ont.	Nagoya University Hospital	Nagoya Japan
Ontario Cancer Foundation Thunder Bay Clinic General Hospital	Port Arthur Ont.	Radiotherapy Aichi Cancer Centre	Nagoya Japan
Ontario Cancer Foundation Windsor Clinic	Windsor Ont.	Cancer Institute Hospital	Tokyo Japan
Metropolitan General Hospital	Windsor Ont.	Jinnah Post Graduate Medical Centre	Lahore Pakistan
Hospital Notre Dame	Montreal Que.	National Taiwan University Hospital	Taipei Taiwan
Jewish General Hospital	Montreal Que.	Provincial Taipei Hospital	Taipei Taiwan
Queen Elizabeth Hospital	Montreal Que.	Veterans General Hospital	Yangmingshan Taiwan
Allan Blair Memorial Hospital	Regina Sask.	Chulalongkorn Hospital and Medical School	Bangkok Thailand
University Hospital	Saskatoon Sask.	Siriraj Hospital and Medical School	Dhomburi Thailand

absorbed dose delivered to each test capsule was to be 500 rad, the participant following his own method of dosimetry exactly as if treating a patient.

Of the two control capsules, one contained unirradiated phosphor, and the other one a sample irradiated to a fixed dose at IAEA prior to being mailed. The unirradiated controls served to detect the background and the irradiated

controls to detect any change in stored luminescence due to unknown heating or other effects on the capsules while in transit. Further irradiated controls retained at IAEA were used to obtain the luminescence decay curves for the phosphors.

The thermoluminescence readings of all capsules were made at the IAEA using a commercial read-out instrument. The phosphor from each capsule gave rise to three readings, the average of which is the measured value for one capsule. Prior to each reading a phosphorescent reference source was inserted into the read out instrument, allowing correction to be made for variations in instrumental sensitivity. The photomultiplier voltage was kept constant at all times. To avoid any error being introduced due to planchet wear and tear, no heating planchet was used more than 12 times.

At the completion of the series a questionnaire was sent to each participant requesting information on the data used by him in determining the administered dose.

Results

Table 2a—2b was prepared on the basis of the information given by the participants in the questionnaire. The various factors used by the participants to calculate absorbed dose from a measured exposure are listed. The first column of the table lists the institute number which was assigned during the intercomparison to prevent identification by any third parties. The other columns show in order the values of the f factor, backscatter factor (BSF), central axis percent depth dose (% DD) and source to surface distance (SSD), tissue air ratio (TAR) and displacement factor (C_D). The information given by the participants indicated that four methods of deriving the absorbed dose from the exposure were in use. The formulas are shown in Table 4.

In order to separate the effects of discrepancies in exposure determination from those due to the factors used in calculation of absorbed dose, uniform values for the various multiplicative terms were adopted as shown in Table 5. It should be noted that while a value of 0.983 was taken for the displacement factor in all cases, the value of this factor is not well known for instruments other than the Farmer-Baldwin thimble ionization chamber. In several cases the kind of measuring chamber used was not reported (institutes 81, 85, 42 and 57). In the last column of Table 2a—2b the normalizing factors are given which represent the correction to the results due to adoption of the uniform values in Table 5.

Table 3a—3b shows the results of the read out evaluations for the test capsules irradiated — as determined by the participants — to an absorbed dose of 500 rad. Since the thermoluminescence readings were taken at any time between 15 and 160 days after irradiation, the results have been normalized to 50 days

Table 2

Factors used by participants to calculate absorbed dose

Table 2a — Canada

Institute No	f rad/R	Field size 10 cm \times 10 cm			C _D	Normalizing factors
		BSF	At 5 cm depth			
			% DD ₅₀	TAR		
71	0.96 ₃	—	—	0.888	1.0	0.996
72	0.97	1.026	78.2 ₀₀	—	1.0	1.008
73	1.0	—	—	0.893	1.0	1.038
74	0.9 ₅₇	—	—	0.893	1.0	0.993
7 ₅	0.96 ₃	1.025	78.5 ₀₀	—	1.0	1.00 ₃
76	0.965	—	—	—	1.0	1.015
77	0.9 ₅₇	1.036	78.5 ₀₀	—	0.985 and 0.98	0.990
78	0.965	—	—	0.893	1.0	1.00 ¹
79	0.9 ₅₇	—	—	0.89 ₃	0.985	0.981
80	0.974	1.026	7 ₅ 9 ₀₀	—	1.0	1.016
81	0.96	—	—	—	1.0	1.010
83	0.9 ₅₇	1.036	75 9 ₀₀	—	0.985	0.993
8 ₄	0.96 ₃	—	—	—	1.0	1.015
86	0.97	1.029	78.2 ₀	—	0.986	0.990

Table 2b — Asia

	Details not available					
42						
43	0.973	1.026	74.0 ₀₀	—	1.0	1.024 and 1.003*
44	1.000	—	74.0 ₀₀	—	1.0	1.073*
4 ₅	0.964	—	—	0.893	1.0	1.001
46	0.97 ₃	1.026	75.9 ₀₀	—	1.0	1.031 and 1.039*
47	0.974	1.026	78.0 ₀₀	—	1.0	1.038*
48	0.960	1.135	74.0 ₀	—	1.0	1.015
49	0.965	1.071	76.0	—	1.0	0.983
50	0.944***	—	—	0.890	—	0.977
51	0.974	—	—	0.890	1.0	0.993
53	0.974	—	—	0.893	1.0	1.011
55	0.960	—	—	0.893	1.0	0.997**
56	0.974	1.026	75.0 ₀₀	—	1.0	1.034*
57						
58	0.965	—	—	0.90 ₃	1.0	1.015

* Includes inverse square law correction ** A cobalt 60 decay correction was also applied

*** Represents C_A

post irradiation using phosphor decay corrections obtained from the irradiated control capsules. The unirradiated control capsules in all cases gave a reading of less than 1 % of the irradiated test capsules. A standard deviation of 2.6 % was found for the irradiated controls.

In Table 3a—3b the first column lists the institute number, next are shown the percentage differences between the mean of all capsule readings for each institute and the overall mean, together with the standard deviation of the institute's mean. The institutional means were recalculated (using the normalizing factors of Table 2a—2b) and the percentage differences between the institute's normalized mean and the new overall mean are given in column 4 of Table 3a—3b. Finally, the last two columns list the make of measuring instrument used by the participant, and the institute's method of absorbed dose calculation according to the formulas shown in Table 4.

Canada. Considering first the factors used by participants in their calculations (Table 2a) it will be noted that there exists some disagreement concerning the f factor: the two values 0.965 and 0.957 occur most frequently, representing the roentgen to rad conversion factor as obtained from ICRU Report 10b (ref. 8) for a photon energy of 1.25 MeV for water and muscle respectively, in earlier ICRU reports these values were given as 0.974 and 0.965, respectively, and hence it is not certain whether the five institutes using 0.965 intended this to be the value for water or for muscle. The value 0.97 was used by two participants, probably representing the earlier ICRU f values. 0.96 occurs once and so does 0.974, the earlier value for water. One participant equated roentgen and rad. The backscatter factor for a 10 cm \times 10 cm cobalt field was taken as 1.026 or 1.025 by three participants, corresponding to the value given in Brit. J. Radiol. Supplement 10 (ref. 4). Two participants adopted the higher value of 1.036 and a value of 1.029 was used in one instance. The per cent depth dose values of 78.5 % at 80 cm SSD and 75.9 % at 60 cm SSD, each used by two participants, correspond to the values given in Supplement 10. 78.2 % at 80 cm SSD was used by two others. The most commonly used value for the tissue air ratio was 0.893 (ref. 4), an earlier value of 0.888, and the value 0.895 occurred in single instances. A displacement factor C_D was used by four participants, of these only two (institutes 77 and 83) made use of the backscatter factor of 1.036 (ref. 3) which requires the use of the factor C_D ; all other participants used the older, slightly lower values of backscatter factor or of tissue air ratio and (except for institutes 79 and 86) ignored the displacement factor, a procedure which is justifiable inasmuch as the effects tend to cancel.

The last column of Table 2a gives the normalizing factors which reflect the disagreement between institutes in their use of the factors involved in calculation.

Table 3

Results of intercomparison of cobalt 60 absorbed dose

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
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Table 3a — Canadian participants

71	2.2	5.3	1.4	N R C	3
72	— 0.1	2.4	0.3	N R C	4
73	— 1.8	3.2	1.5	Victoreen	3
74	4.8	4.3	3.7	Farmer S S	3
75	— 3.5	3.8	— 3.4	Victoreen	4
76	— 6.8	3.9	— 5.8	Victoreen	1
77	— 2.7	2.6	— 4.1	Farmer S S	4 and 1
78	2.7	4.3	2.5	Victoreen	3
79	3.1	2.8	0.7	Farmer S S	3
80	— 1.2	5.0	0.0	N R C	4
81	3.1	3.0	3.7	Ionization Chamber	1
83	2.5	2.3	1.4	Farmer S S	4
85	— 3.1	2.7	— 2.1	?	1
88	1.1	2.9	0.3	N R C	4

Standard deviation 3.3 % of mean 2.9 % of mean

Extreme difference 11.6 % of mean 9.5 % of mean

The two means differ by 0.4 %.

* Not recalculated ** Baldwin Ionec = Baldwin Ionec ionization meter Mk 3 Baldwin Reference = Baldwin Farmer electrometer with reference chamber Farmer S S = Farmer Baldwin secondary standard dosimeter N R C = National Research Council of Canada reference instrument (aluminum cavity ionization chamber with Townsend balance electrometer) Radocon = Victoreen dosimeter Siemens = Siemens dosimeter Victoreen = Victoreen condenser R meter (25 R thimble chamber)

of absorbed dose from a measured exposure. It is seen that these are quite small, generally less than 1 %, with a maximum of 3.8 % for the participant using an f factor of 1.0.

Turning next to Table 3a, it is seen that the agreement between institutes with respect to absorbed dose (column 2) is better than 5 % for all except one participant, with maximum deviations from the mean of +4.8 % and —6.8 %, the individual standard deviations also being 5 % or less with one exception. Although the normalizing factors, as mentioned, are generally small, it is seen

Table 3 (cont.)

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
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Table 3b — As an participants

49	-16.3	4.3	-17.3	?	?
43	-8.6	3.1	-8.7	Farmer S S	1 and 4
44	-5.0	10.8	0.5	Victoreen	2
45	-6.1	7.9	-7.0	Farmer S S	3
46	7.4	3.5	9.7	Baldwin Ionex	1 and 4
47	5.3	2.5	8.1	Victoreen	4
48	-1.1	4.4	-0.7	Baldwin Ionex	4
49	6.6	3.9	3.7	Victoreen	4
50	3.3	3.9	-0.7	Farmer S S	3
51	6.4	5.7	4.6	Frederick and Radocon	3
52	7.6	1.5	7.5	Baldwin Reference	3
53	15.4	2.0	13.8	Siemens	3
56	-10.8	9.1	-8.9	Siemens	4
57	9.9	3.8	1.8	?	?
58	-7.3	4.0	-7.0	Farmer S S	3

Standard deviation 8.7 of mean 8.4 of mean

Extreme difference 31.7 of mean 31.1 of mean

The two means differ by 1.1

(column 4) that the percent deviations from the normalized mean are reduced for eleven out of fourteen participants the maximum deviations from the mean changing to +3.7% and -5.8% with a corresponding small decrease in the standard deviation expressed as a percentage of the mean. The differences among institutes shown in column 4 now correspond chiefly to differences in exposure determination and are seen to be less than 4% in the great majority of cases.

Participants used three kinds of measuring instruments with numbers equally divided between the Farmer and NRC secondary standard and the Victoreen R meter. It is noteworthy that the four institutes employing the reference instrument provided by the National Research Council of Canada especially for cobalt 60 beam calibrations (ref. 1) had some of the smallest per cent deviations. For two institutes details of the instruments used were not available.

Table 3a further shows that three methods of dose calculation were used by the participants. Methods 3 and 4 both involving exposure measurements in air

Table 3

Results of intercomparison of cobalt 60 absorbed dose

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
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71	2.2	5.3	1.4	N R C	3
72	— 0.1	2.4	0.3	N R C	4
73	— 1.8	3.2	1.5	Victoreen	3
74	— 4.8	4.3	3.7	Farmer S S	3
75	— 3.5	3.8	— 3.4	Victoreen	4
76	— 6.8	3.9	— 5.8	Victoreen	1
77	— 2.7	2.6	— 4.1	Farmer S S	4 and 1
78	2.7	4.3	2.5	Victoreen	3
79	3.1	2.8	0.7	Farmer S S	3
80	— 1.2	5.0	0.0	N R C	4
81	3.1	3.0	3.7	Ionization Chamber	1
83	2.5	2.3	1.4	Farmer S S	4
84	— 3.1	2.7	— 2.1	?	1
86	1.1	2.9	0.3	N R C	4

Standard deviation 3.3 % of mean 2.9 % of mean

Extreme difference 11.6 % of mean 9.5 % of mean

The two means differ by 0.4 %.

* Not recalculated ** Baldwin Ionex = Baldwin Ionex ionization meter Mk. 3 Baldwin Reference = Baldwin Farmer electrometer with reference chamber Farmer S S = Farmer Baldwin secondary standard dosimeter N R C = National Research Council of Canada reference instrument (aluminum cavity ionization chamber with Townsend balance electrometer) Radcon = Victoreen dosimeter Siemens = Siemens dosimeter Victoreen = Victoreen condenser R meter (25 R thimble chamber)

of absorbed dose from a measured exposure. It is seen that these are quite small, generally less than 1 %, with a maximum of 3.8 % for the participant using an f factor of 1.0.

Turning next to Table 3a, it is seen that the agreement between institutes with respect to absorbed dose (column 2) is better than 5 % for all except one participant, with maximum deviations from the mean of +4.8 % and —6.8 % the individual standard deviations also being 5 % or less with one exception. Although the normalizing factors as mentioned, are generally small, it is seen

Table 3 (cont.)

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
Table 3b — Asian participants					
42	-16.5	4.3	-17.3*	?	?
43	-8.6	3.1	-8.7	Farmer III S	1 and 4
44	-5.0	10.8	0.5	Victoreen	2
45	-6.1	7.9	-7.0	Farmer S S	3
46	7.4	3.5	9.7	Baldwin Ionex	1 and 4
47	5.3	2.5	8.1	Victoreen	4
48	-1.1	4.4	-0.7	Baldwin Ionex	4
49	6.6	3.9	3.7	Victoreen	4
50	3.3	3.9	-0.2	Farmer S II	3
51	6.4	5.7	4.6	Frucke and Radocon	3
53	7.6	1.5	7	Baldwin Reference	3
55	15.4	2.0	13.8	Siemens	3
56	-10.8	9.1	-8.9	Siemens	4
57	2.9	3.8	1.8	?	?
58	-7.3	4.0	-7.0	Farmer S S	3

Standard deviation 8.7 of mean 8.4 of mean

Extreme difference 31.7 of mean 31.1 of mean

The two means differ by 1.1

(column 4) that the percent deviations from the normalized mean are reduced for eleven out of fourteen participants the maximum deviations from the mean changing to +3.7% and -5.8% with a corresponding small decrease in the standard deviation expressed as a percentage of the mean. The differences among institutes shown in column 4 now correspond chiefly to differences in exposure determination and are seen to be less than 4% in the great majority of cases.

Participants used three kind of measuring instruments with numbers equally divided between the Farmer and NRC secondary standard and the Victoreen R meter. It is noteworthy that the four institutes employing the reference instrument provided by the National Research Council of Canada especially for cobalt 60 beam calibrations (ref. 1) had some of the smallest per cent deviations. For two institutes details of the instruments used were not available.

Table 3a further shows that three methods of dose calculation were used by the participants. Methods 3 and 4 both involving exposure measurements in air

Table 4

Formulas for calculation of absorbed dose from measured exposure by different methods

Method

1	$D_s = f C_D w \lambda_s$
2	$D_s = f C_D w \lambda_{0.5} P_s$
3	$D_s = f C_D \lambda_s T_s$
4	$D_s = f C_D \lambda_{0.5} P_s B$
D_s	Absorbed dose in water at (SSD + 5) cm i.e. at position of LiF capsule
$w \lambda_s$	Exposure measured in water at (SSD + 5) cm
$w \lambda_{0.5}$	Exposure measured in water at (SSD + 0.5) cm
λ_s	Exposure measured in air at (SSD + 5) cm
$\lambda_{0.5}$	Exposure measured in air at (SSD + 0.5) cm
f	Rad per roentgen factor (rad/R)
C_D	Displacement factor
P_s	Central axis fractional depth dose at 5 cm depth
T_s	Tissue air ratio at 5 cm depth
B	Backscatter factor
SSD	Source to-surface distance (cm)

were followed by a majority, whereas method 1, based on measurements in water, (as recommended by ICRU and the 'H P A Code of Practice'), was used by three participants only (76, 81 and 85). This method, for purposes of this inter-comparison, does not involve backscatter, per cent depth dose or tissue air ratio, requiring two factors only (in addition to the chamber quality factor and — for unsealed ionization chambers — the temperature pressure factor). The three institutes employing this method did not make use of a displacement factor, however. Method 4, although calling for the largest number of multiplicative terms, was used by six institutes. One participant used methods 4 and 1 as checks against one another.

As in Table 2b shows that various f factors are in use, the value preferred by six participants clustering around 0.974, which is the older value for water, three participants used 0.965 or 0.964, one chose 0.96, and an unusually low value of 0.944 (this is probably the C_a factor, which includes f and C_D to be used with method No. 1 as recommended in 1964 in the H P A Code of Practice for dosimeters calibrated at an X-ray energy of 2 MV) occurs in one instance. One participant did not report a value for this factor, and it is assumed that conversion to rad was not carried out. Details were not available for two institutes. The backscatter factor given in the depth dose tables (ref. 4) was used in most cases, with one participant (48) employing the higher value of 1.035. The per cent depth doses quoted are for shorter distances than in Canada, and while values for 50 and 60 cm SSD agree with those given in

Table 5

Values adopted for purposes of normalization — Factors applying to cobalt 60 10 cm × 10 cm field 5 cm depth — * Values of P_d for 55 cm and 70 cm SSD were calculated according to eq (13) in reference (6)

			SSD cm	\bar{M} (ref 4)
f (water) rad/R	0.965	(ref 8)	50	0.740
C_D	0.985	(ref 4)	55	0.750*
T	0.905	(ref 5)	60	0.759
\bar{M}	1.035	(ref 5)	70	0.774*
Half life	5.76 y	(ref 9)	80	0.785

ref 4 the values at 70 cm SSD are discrepant. The tissue air ratios again correspond closely to those given in ref 4 with one exception (58) where the higher value of 0.905 (ref 5) was used. It must be noted that the two institutes (48 and 58) using the higher values of backscatter and of tissue air ratio failed as was the case in Canada to make use of a displacement factor. One participant (51) appears to have obviated the use of this factor by basing his calibration on measurements with a Fricke dosimeter.

The majority of Asian participants used the older slightly lower values of the factors and none of them applied a displacement factor. The normalizing factors tend to be larger for these participants and in five instances (43, 44, 46, 47 and 56) include small corrections for inverse square law terms omitted by the participant. In one instance (55) radioactive decay calculations appeared faulty and were corrected for purposes of normalization by an additional factor not included in the listed normalizing factors.

Considering next Table 3b it is seen that disagreement between institutes with respect to absorbed dose (column 2) reaches a maximum of about 32% (+15.4% and -16.3%, respectively) with a standard deviation of about 9%. Large individual deviations from the mean are found for three institutes in particular. Only four participants have deviations from the mean of 5% or less as compared to only one having a deviation of more than 5% for Canada. As a result of using the normalizing factors some reductions do occur in the per cent deviations in nine instances (column 4) but in the remaining six the results were less satisfactory.

A greater variety of measuring instruments were used by the Asian participants with the Farmer secondary standard being slightly in the lead over the Victoreen chamber followed by Baldwin Ionex Siemens dosimeter, Baldwin reference condenser chamber and Victoreen Radocon used in combination with a Fricke dosimeter.

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Formulas for calculation of absorbed dose from measured exposure by different methods

Method	
1	$D_s = f C_D w \lambda_s$
2	$D_s = f C_D w \lambda_{0.5} P_s$
3	$D_s = f C_D \lambda \lambda_s T_s$
4	$D_s = f C_D \lambda \lambda_{0.5} P_s B$
D_s	Absorbed dose in water at (SSD + 5) cm i.e. at position of LiF capsule
$w \lambda_s$	Exposure measured in water at (SSD + 5) cm
$w \lambda_{0.5}$	Exposure measured in water at (SSD + 0.5) cm
$\lambda \lambda_s$	Exposure measured in air at (SSD + 5) cm
$\lambda \lambda_{0.5}$	Exposure measured in air at (SSD + 0.5) cm
f	Rad per roentgen factor (rad/R)
C_D	Displacement factor
P_s	Central axis fractional depth dose at 5 cm depth
T_s	Tissue air ratio at 5 cm depth
B	Backscatter factor
SSD	Source to surface distance (cm)

were followed by a majority, whereas method 1, based on measurements in water, (as recommended by ICRU and the 'H.P.A. Code of Practice'), was used by three participants only (76, 81 and 85). This method, for purposes of this inter-comparison, does not involve backscatter, per cent depth dose or tissue air ratio, requiring two factors only (in addition to the chamber quality factor and — for unsealed ionization chambers — the temperature pressure factor). The three institutes employing this method did not make use of a displacement factor, however. Method 4, although calling for the largest number of multiplicative terms, was used by six institutes. One participant used methods 4 and 1 as checks against one another.

Asia. Table 2b shows that various f factors are in use, the value preferred by six participants clustering around 0.974, which is the older value for water, three participants used 0.965 or 0.964, one chose 0.96, and an unusually low value of 0.944 (this is probably the C_1 factor, which includes f and C_D , to be used with method No. 1 as recommended in 1964 in the H.P.A. Code of Practice for dosimeters calibrated at an X-ray energy of 2 MV) occurs in one instance. One participant did not report a value for this factor, and it is assumed that conversion to rad was not carried out. Details were not available for two institutes. The backscatter factor given in the depth dose tables (ref. 4) was used in most cases, with one participant (48) employing the higher value of 1.035. The per cent depth doses quoted are for shorter distances than in Canada, and while values for 50 and 60 cm SSD agree with those given in

Table 5

Values adopted for purposes of normalization—Factors applying to cobalt 60 10 cm × 10 cm field 5 cm depth—* Values of P_A for 55 cm and 70 cm SSD were calculated according to eq (13) in reference (6)

			SSD cm	F (ref 4)
f (water) rad/R	0.960	(ref 8)	50	0.740
C _D	0.985	(ref 4)	55	0.750*
T	0.905	(ref 5)	60	0.759
B	1.035	(ref 5)	70	0.774*
Half life	5.26 y	(ref 9)	80	0.785

ref 4 the values at 70 cm SSD are discrepant. The tissue air ratios again correspond closely to those given in ref 4 with one exception (58) where the higher value of 0.905 (ref 5) was used. It must be noted that the two institutes (48 and 58) using the higher values of backscatter and of tissue air ratio failed as was the case in Canada to make use of a displacement factor. One participant (51) appears to have obviated the use of this factor by having his calibration on measurements with a Fricke dosimeter.

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Formulas for calculation of absorbed dose from measured exposure by different methods

Method

1	$D_s = f C_D w \Lambda_s$
2	$D_s = f C_D w \Lambda_{0.5} P_s$
3	$D_s = f C_D \Lambda \Lambda_s T_s$
4	$D_s = f C_D \Lambda \Lambda_{0.5} P_s B$
D_s	Absorbed dose in water at (SSD + 5) cm i.e. at position of LiF capsule
$w \Lambda_s$	Exposure measured in water at (SSD + 5) cm
$w \Lambda_{0.5}$	Exposure measured in water at (SSD + 0.5) cm
$\Lambda \Lambda_s$	Exposure measured in air at (SSD + 5) cm
$\Lambda \Lambda_{0.5}$	Exposure measured in air at (SSD + 0.5) cm
f	Rad per roentgen factor (rad/R)
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were followed by a majority, whereas method 1, based on measurements in water, (as recommended by ICRU and the 'H P A Code of Practice'), was used by three participants only (76, 81 and 85). This method, for purposes of this intercomparison, does not involve backscatter, per cent depth dose or tissue air ratio, requiring two factors only (in addition to the chamber quality factor and — for unscaled ionization chambers — the temperature pressure factor). The three institutes employing this method did not make use of a displacement factor, however. Method 4, although calling for the largest number of multiplicative terms, was used by six institutes. One participant used methods 4 and 1 as checks against one another.

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			SSD cm	P (ref 4)
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C_D	0.985	(ref 4)	55	0.70*
T	0.905	(ref 5)	60	0.759
II	1.035	(ref 5)	70	0.774*
Half life	5.76 y	(ref 9)	80	0.785

ref 4 the values at 70 cm SSD are discrepant. The tissue air ratios again correspond closely to those given in ref 4, with one exception (58) where the higher value of 0.905 (ref 5) was used. It must be noted that the two institutes (48 and 58) using the higher values of backscatter and of tissue air ratio failed as was the case in Canada to make use of a displacement factor. One participant (51) appears to have obviated the use of this factor by basing his calibration on measurements with a Fricke dosimeter.

The majority of Asian participants used the older, slightly lower values of the factors and none of them applied a displacement factor. The normalizing factors tend to be larger for these participants and in five instances (43, 44, 46, 47 and 56) include small corrections for inverse square law terms omitted by the participant. In one instance (55) radioactive decay calculations appeared faulty and were corrected for purposes of normalization by an additional factor not included in the listed normalizing factors.

Considering next Table 3b it is seen that disagreement between institutes with respect to absorbed dose (column 2) reaches a maximum of about 32% (+15.4% and -16.3% respectively) with a standard deviation of about 9%. Large individual deviations from the mean are found for three institutes in particular. Only four participants have deviations from the mean of 5% or less as compared to only one having a deviation of more than 5% for Canada. As a result of using the normalizing factors some reductions do occur in the per cent deviations in nine instances (column 4) but in the remaining six the results were less satisfactory.

A greater variety of measuring instruments were used by the Asian participants with the Farmer secondary standard being slightly in the lead over the Victoreen chamber followed by Baldwin Ionex Siemens dosimeter, Baldwin reference condenser chamber and Victoreen Radocon used in combination with a Fricke dosimeter.

Finally it is seen that most participants also preferred methods involving in air exposure measurements (methods 3 and 4). Method 1 was used in two instances only, both times in combination with method 4, and method 2 was used in a single instance.

Discussion

The results for Canada are similar to those of the first IAEA postal dose intercomparison (ref. 7) which showed extreme differences between institutes of 14.3 % and 9.3 % with respect to absorbed dose and normalized dose, respectively. The present results give a value of 11.6 % for the extreme difference between institutes with respect to absorbed dose, and a corresponding figure of 9.5 % with respect to the normalized values. This residual difference of about 10 % between institutes appears to be larger than is desirable, but is, of course, considerably better than the results found for the Asian participants where the differences reach up to 30 %. Some of the Asian institutes are certainly doing careful work in every way comparable to that done in other parts of the world but other, perhaps more isolated clinics, have greater difficulties in following modern procedures and maintaining good calibrations.

It is clear, in any case, that only a small part of the differences between clinics is due to the factors used to convert exposure to absorbed dose. Thus a basic improvement is only likely to result from (1) maintaining properly calibrated, reliable dosimeters in each clinic, (2) adopting a reproducible dosimetric procedure (e.g. using a standard calibration phantom), and (3) using accepted factors to calculate absorbed dose from exposure. It should be noted that if method 1 is adopted this requires agreement on two factors only (f and C_D), and the user may select whatever per cent depth dose values (or tissue air ratios) he feels are most appropriate for his conditions to calculate doses at any other points.

It appears that the procedure — equivalent to method 1 — recommended by ICRU (ref. 2) for calibration is not yet used to any large extent, nor is the need for a displacement factor well understood. In methods 3 and 4, which are at present widely used, the displacement factor is necessary as a consequence of the definition of tissue air ratio (and, by implication, of backscatter factor) in terms of a ratio of two absorbed doses.

It should be noted that the factor C_D , which occurs in all four methods listed in Table 4, has two different derivations depending on whether (1) an exposure is measured inside a medium such as water (methods 1 and 2), or (2) an exposure is measured in air and then converted to absorbed dose in water or soft tissue (as in methods 3 and 4). For case (1) only, the correction factor C_D will vary with the dimensions and construction of the measuring chamber, being

due to an actual displacement of medium owing to the introduction of the chamber for case (2) the factor C_D is not a function of the physical presence of a measuring chamber but arises from the concept of a volume of phantom material just large enough to provide the maximum electronic build up at the point of measurement (definition of tissue air ratio, ref 2 page 5), and is thus a function of the radiation energy only. It is fortunate that for chambers such as the Farmer-Baldwin thimble chamber, at any rate, the values of the two factors seem to be nearly identical.

Finally, in conclusion it may be stated that the postal intercomparison of absorbed dose using LiF dosimeters as instituted by the IAEA has proven to be practical and effective in helping to evaluate and improve accuracy of dose delivery in teletherapy. During the past year this technique has been further standardized and refined and is being made available by IAEA to an increasing number of radiotherapy centres. There is every indication that this IAEA service is having a significant effect towards achieving international consistency and comparability of radiation dosage in the treatment of cancer.

Acknowledgements

The authors are indebted to Dr W. H. Henry (NRC Ottawa) and Dr R. Loevinger (NBS Washington) for helpful comments and are pleased to acknowledge the valuable assistance of S. Malo Alvarez and S. Tsialas in the preparation and evaluation of phosphor samples. The work described here was carried out while the senior author (P.M.P.) was Head of the Dosimetry Section, International Atomic Energy Agency, Vienna, Austria, on leave of absence granted by the Ontario Cancer Treatment and Research Foundation, Toronto, Canada. The views expressed are those of the authors and not necessarily those of the International Atomic Energy Agency.

SUMMARY

A series of dosimeter capsules containing LiF phosphor were distributed by the International Atomic Energy Agency to 14 Canadian radiotherapy centres and to 15 centres in six countries in Asia. The dosimeters were irradiated with a cobalt 60 beam at a depth of 5 cm in water to an absorbed dose calculated by the participants to be 500 rad and returned to IAEA by mail for read-out. The maximum difference in absorbed dose between participating centres was found to be 11.6% for Canada and 31.7% for the Asian countries with per cent standard deviations of 3.3% and 8.7% for the respective overall mean doses. The lack of uniformity in the dosimetric methods and in the factors used by participants to convert exposure to absorbed dose indicates a need for closer international collaboration and standardization in medical radiation dosimetry.

ZUSAMMENFASSUNG

Eine Reihe von mit LiF-Phosphor gefüllten Dosimeterkapseln wurde von der Internationalen Atomenergiebehörde (IAEO) an 14 Radiotherapie-Zentren in Kanada sowie an

15 Zentren in sechs asiatischen Ländern versandt. Den Dosimetern wurde in einer Tiefe von 5 cm Wasser eine von den Teilnehmern berechnete Energiedosis von 500 rad Kobaltstrahlung verabreicht. Zur Auswertung wurden die Dosimeter per Post an die IAEA retourniert. Es ergab sich eine zwischen verschiedenen Teilnehmern bestehende Maximaldifferenz in Energiedosis von 11.6 % in Kanada, bzw. 31.7 % in Asien mit einer prozentualen Standardabweichung für die Durchschnittswerte von 3.3 %, bzw. 8.7 %. Die verschiedenen von den Teilnehmern benutzten dosimetrischen Methoden sowie Faktoren zur Umrechnung von Bestrahlungen auf Energiedosis weisen auf die Notwendigkeit hin enger internationaler Zusammenarbeit und Standardisierung in der medizinischen Strahlendosimetrie zu fordern.

RÉSUMÉ

Une série de capsules dosimétriques contenant des scintillateurs de LiF ont été distribuées par l'Agence Internationale de l'Energie Atomique à 14 centres de radiothérapie canadiens et à 15 centres situés dans 6 pays asiatiques. Les dosimètres ont été irradiés par le rayonnement du cobalt 60 à une profondeur de 5 cm dans l'eau jusqu'à une dose absorbée calculée par les participants comme étant égale à 500 rad et les dosimètres ont été retournés à l'IAEA par la poste pour lecture de la dose. Le maximum de différence de doses absorbées entre les différents centres participant à l'expérience a été de 11.6 % pour le Canada et de 31.7 % pour les pays asiatiques avec des déviations standard de 3.3 % et de 8.7 % pour les doses moyennes respectives. Le manque d'uniformité dans les méthodes dosimétriques et dans les facteurs utilisés par les participants pour convertir la dose d'exposition en dose absorbée indique la nécessité d'une collaboration internationale plus étroite et d'une standardisation dans la dosimétrie des radiations en médecine.

REFERENCES

1. AITKEN J. H. and HENRY W. H. A portable apparatus for measuring exposure in roentgens from strong gamma ray sources. *Int J appl Radiat* 14 (1963) 511.
2. CLINICAL DOSIMETRY. ICRU Report 10d. NBS Handbook No. 87. National Bureau of Standards. Washington D. C. 1963.
3. CUNNINGHAM J. R., GUPTA S. K. and JOHNS H. E. An examination of the definition and magnitude of back scatter factor for cobalt 60 gamma rays (letter). *Brit J Radiol* 38 (1965) 637.
4. DEPTHI DOSE TABLES FOR USE IN RADIOTHERAPY. *Brit J Radiol* (1961) Suppl. No. 10.
5. GUPTA S. K. and CUNNINGHAM J. R. Measurement of tissue air ratios and scatter functions for large field sizes for cobalt 60 gamma radiation. *Brit J Radiol* 39 (1966) 7.
6. PFALZNER I. M. The variation of axial depth dose with focal distance with field area and with depth. *Brit J Radiol* 34 (1961) 236.
7. — and MALO ALVAREZ S. Intercomparison of absorbed dose in cobalt 60 teletherapy using mailed LiF dosimeters. *Acta radiol Ther Phys Biol* 7 (1968) 379.
8. PHYSICAL ASPECTS OF IRRADIATION. ICRU Report 10b. NBS Handbook No. 85. National Bureau of Standards. Washington D. C. 1963.
9. (THE) RADIOCHEMICAL MANUAL. Second edition. Radiochemical Centre. Amersham. Bucks. 1966.

OBJECTIVE PROCEDURE OF CONSTRUCTING STAGES IN CANCER

Its application in recurrent carcinoma of the corpus uteri

by

SAMUEL S. KUROHARA and FREDERICK W. GEORGE III

Staging has been an empirically developed procedure by which clinical oncologists have tried to classify a cancer entity into levels of advancement these may then be used as a means of predicting the behaviour of cancer in an individual or in groups of patients. The clinical variables most commonly employed in the development of such classifications have been the anatomic sites and the extent of involvement. Less often used are certain other of the characteristics of the disease.

A functionally satisfactory system of clinical cancer staging should (1) separate patients with varyingly advanced lesions into distinct groups each group possessing (a) a consistently predictable and different level of prognosis and constituting (b) a roughly equal percentage of the total patient population (2) be simple enough to be widely applicable (3) provide an effective and reliable means of comparing different methods of treatment and (4) consistently predict the outcome of the disease in individual patients taking into account alterations induced by additional variables such as methods of treatment.

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REFERENCES

1. AITKEN, J. H. and HENRY, W. H. A portable apparatus for measuring exposure in roentgens from strong gamma ray sources. *Int. J. appl. Radiat.* 14 (1963) 511.
2. CLINICAL DOSIMETRY. ICRU Report 10d. NBS Handbook No. 87. National Bureau of Standards, Washington D. C. 1963.
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6. PFALZNER, P. M. The variation of axial depth dose with focal distance with field area and with depth. *Brit. J. Radiol.* 34 (1961) 236.
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part of a more extensive study on clinical features and prognoses in 300 cases of recurrent carcinoma of the corpus uteri (KUROHARA et coll 1969) treated at the Roswell Park Memorial Institute

Measurements Assessment of the clinical stage of the malignant process in this group of patients and in any group so utilized should be performed by methods generally applicable to all patients in the sample population. These ordinarily include the recording of the medical history, and the physical examination and laboratory tests at the time of admission. Biopsy of the lesion, complete blood count, urinalysis, fasting blood sugar, blood urea nitrogen, venereal disease and liver function tests, and chest and metastatic survey roentgenograms will probably be the rule in all cases considered to have cancer. Certain special procedures of particular importance in the specific type of cancer being considered are almost always employed in most medical centers. For example, a pelvic examination under anesthesia with a diagnostic curettage of the uterine cavity is almost invariably carried out in cases of gynecologic cancer, while urography is usual in cancer of the cervix or testis, and paranasal sinus roentgenograms in cases of carcinoma of the antrum.

In the present study, assessment of the extent of involvement of the anatomic sites was obtained by the above mentioned routine examinations. This had been carefully recorded in the medical records of the cases either by word or by diagram. Biopsy specimens and roentgenograms were reviewed.

The anatomic sites of potential involvement by recurrent cancer were defined as nearly as the available data permitted. Pelvic organs or regions directly visible or palpable were subdivided into segments. For example, the vagina was subdivided into upper and lower halves (introitus and minor lip being included in the latter). The upper part of the vagina was subdivided into anterior, posterior, right lateral and left lateral walls, and the apex or cuff. The lower part of the vagina was similarly subdivided, excepting the last mentioned subdivision. The true pelvis was subdivided into regions surrounding the vagina on the right and left aspects, as well as left superiorly, and into regions close to the pelvic wall bilaterally. Lesions involving the latter sites were considered 'fixed to the pelvic wall'.

Distant sites of spread were less discretely defined than the local or regional sites. For example, abdominal involvement was designated by the findings of a mass in the right upper quadrant, midabdomen or elsewhere, or of multiple masses or ascites. Thoracic sites were designated as lung, pleura and mediastinum. Skeletal sites as pelvis, spine, ribs.

Each of the anatomic sites were identified numerically. These numerical scores were punched on IBM cards for computer purposes. Zero scores were reserved

The definitions and other criteria that have established the advancement levels and related guidelines in most existing staging systems have to date been derived by more or less a trial and error method. In a somewhat arbitrary manner, individuals or committees have proposed, considered and perhaps adopted certain patterns of anatomic spread, or extent of involvement, as hopefully valid indices by which to predict the outcome and thus establish the prognosis. These empirically constructed staging systems have been produced in an almost endless number of variations for virtually every type of cancer. However, on careful evaluation only a handful of them can be shown to meet the criteria for a satisfactorily functioning clinical cancer staging system.

The major cause of the general ineffectiveness of cancer staging systems has been the lack of careful analysis, prior to the development of the system, to identify the clinical factors essential to a proper staging. Such identified factors should always correlate with the outcome of the disease in similar patient populations. An arbitrary selection of these important components cannot ensure categorization of patients into well defined groups. If the prognostic distinctions of each of the component clinical factors are not identified before combining them into the various levels of stage, a mixing of factors with different degrees of prognostic distinctions within the same stage will inevitably occur. Each such mixing may conceal or distort important patient information and play a role in concentrating disproportionate numbers of patients to one or two levels of staging, thus producing an imbalanced system.

The following method is advanced as a logical and objective approach to the problem of constructing staging systems and may be summarized as follows:

Variables (or clinical patient factors) are provisionally selected as representing (1) valid measures of prognosis in a specific variety of cancer, and (2) findings readily obtainable under ordinary clinical conditions, decomposed as discretely as possible into their essential ingredients.

Survival rates of each separate subset of patients manifesting all the variables, selected either alone or in combinations, are computed from substantial clinical experience by means of appropriate statistical techniques.

Subsets with one or more factors having similar survival characteristics are then combined into a few levels or categories each involving approximately equal numbers of the patient population analyzed and each association with distinctly prognostic features.

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The construction of a cancer staging system is illustrated by a series of 149 cases of endometrial carcinoma recurrent after total hysterectomy. The material is

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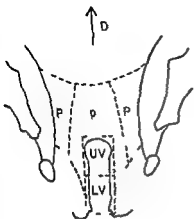


Fig 2 Anatomic sites of involvement UV—upper vagina LV—lower vagina p—pelvis (not fixed to pelvic wall) P—pelvis (fixed to pelvic wall) D—distant

statistical computation could have been used a special computer program (BASCO & KUROHARA in preparation) designed to generate a matrix of simple or complex or both variables and to perform analysis was employed in order to save time and labor and lessen the chances of human error common in manual methods

A schematic diagram is shown in Fig 1 for the matrix of bivariate distribution of scores produced by the program Variables having any of three kinds of measured scores can be handled in the columns and rows Those having *continuous* scores (e.g. age uterine cavity depth survival time) those having *ordinal* scores (e.g. histologic grade clinical stage gravida), and those having *nominal* scores (e.g. sex color histology treatment categories) can be tabulated against each other like against like or like against unlike score type variables

The superscript *a* is used to indicate that partitioning of original scores of one or more variables can be degenerated into ordered or non ordered scores with uniform or non uniform intervals of the original These can be combined with ordinal or nominal or both scores of other kinds of variables to produce a new complex nominal variable in the column or rows or both For example, the first column may be a symptom complex group into which are incorporated all patients 25 to 45 years of age with 2 to 7 months symptom duration and with a hemoglobin of 8 to 10 mg % the second column a symptom complex group including those of 60 to 65 years with 0 to 2 months symptom duration with a hemoglobin of 10 to 15 mg % and with no previous history of surgery The various levels of histologic grade of cancer or levels or classes of some other complex variable may be assigned in the rows The relative frequency of distribution for the entire table for column or rows or both can be obtained by option

		CROSS TABULATION			
		COLUMN			
CONTINUOUS ORIGINAL NOMINAL	SCALE	ORDER			
		1	2	3	4
		CLASS I $V + V_2$ $V_1^2 + V_2^2$	II $V^2 + V_2^2 + V_3^2$ $+ V_2^2$	III $V^2 + V_3^2$ $+ V_2^2$	IV $V^2 + V_3^2 + V_4^2$ $+ V_2^2 + V_3^2$
ROW	CLASS I				
	II				
	III				
	IV				
		GENERAL SELECTOR VARIABLES			
		V_1^2	V_2^2	V_3^2	V_4^2
		$V_1^2 + V_2^2$	$V_2^2 + V_3^2$	$V_3^2 + V_4^2$	$V_4^2 + V_5^2$

Fig. 1 Matrix of bivariate distribution of scores V_b^a —bth variable $a=1, 2, 3, \dots$ nth intervals of a new score into which the original values of the bth variable can be partitioned. O—absence of new score.

for absence of involvement in a particular anatomic segment, and nines for no information. Numbers one through eight were used for the subdivisions of the anatomic segments involved.

The choice of the degree of discreteness in defining anatomic sites or segments and in subdividing them, depends on many factors. It is usually governed by conventionally defined anatomic parts of organs or regions. It also depends on how well the clinicians have been able to distinguish involvement between the variously defined sites by physical examination and through routinely performed laboratory procedures. Divisions that are too fine lead to unreliable and overlapping measurements and to undesirable excessive dilution of the material. Divisions that are too rough produce loss of important information.

The choice of properties and categories for analysis is an intellectual exercise requiring the highest order of clinical judgment, as essential in this method for constructing staging systems as in any other. It can only be made by physicians who are exceptionally well grounded in oncology and particularly familiar with the cancer entity under consideration. However, the decisions made are provisional and in this method are subjected to quality control evaluation. Furthermore, the method establishes the hierarchy of the properties selected in a way independent of clinical judgment.

Computation. Analysis of data on cards were undertaken on the 360-40 IBM computer. Although manual procedures of data sorting, manipulation and

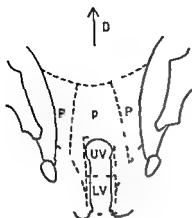


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






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Table 1

**Anatomical classification of recurrent endometrial carcinoma — For symbolic concepts refer to FEINSTEIN (1967)*

Diagram	Definition	Symbolism	Survival
		STAGE I	
	Upper Vagina	UV LV p F D	45/55 (82)
		STAGE II A	
		SUBTOTAL	16/30 (53)
	Pelvis (not fixed)	UV LV p F D	2/3 (66)
	Lower Vagina	UV LV p F D	8/11 (73)
	Upper Vagina and Pelvis (not fixed)	UV LV p F D	6/16 (38)
		STAGE II B	
		SUBTOTAL	7/32 (22)
	Upper Vagina and Pelvis (fixed)	UV LV (p P) F D	4/9 (44)
	Lower Vagina and Pelvis (not fixed)	UV LV p F D	2/6 (33)
	Pelvis (fixed)	UV LV (p P) F D	1/9 (11)
	Upper and Lower Vagina	UV LV p F D	0/4 (0)
	Upper and Lower Vagina and Pelvis (not fixed)	UV LV p F D	0/4 (0)
		STAGE III	
		SUBTOTAL	5/34 (15)
Various	Diagnosis	(UV LV UV LV) p F D (UV LV UV LV+UV LV) (p P p P) D+UV LV (p P p P) D UV LV F D	73/143 (51)
		TOTAL	

* Not all types of anatomical configurations are shown

** For abbreviations refer to Fig 2 ■ Symbol + indicates either or or both symbol both and symbol UV negation = upper vagina not involved

*** Crude 32 month survival rate

The selection procedure operable in columns, rows, or both, can be applied to all cases to be included in the table. However, in addition to manual selection, this can be done automatically on all combinations of a specified number of variables. For example, in a set of four binary (yes or no) variables, tabulations of 16 types of combination of cases are produced.

they were used singly or in combinations. In addition, many of them were not represented by the case material, many subsets being found empty. These latter were eliminated and those remaining were grouped together according to similarities in survival rates after detection of recurrences. The distant group contains many subgroups of cases with various patterns of spread, all of which were in distinct. However, once the presence of cancer was established outside the pelvis, the prognosis was predictably poor.

A summary of representative patterns of recurrences is given in Table 1. The arrangement is in decreasing order of crude 32 month survival rates. This rate has been substituted for the one of 5 year survivals, for reasons that will be taken up in the discussion of Fig. 3.

The stage I level includes only cases of pure upper vaginal recurrences with a 32 month survival rate of 82 %. Stage II includes cases with lower vaginal or pelvic recurrences. The survival rate is 39 %. This stage has been divided into two levels at a point where the subgroup with the highest rate contained cases with pelvic fixation. Because of the limited number of cases this point of demarcation was chosen more from a morphologic than a statistical context so that this choice had to be somewhat arbitrary. It is seen that stage II A cases are those with central pelvic recurrences with or without upper vaginal lesions having a 32 month survival rate of 53 %. Stage II B cases are those exhibiting pelvic fixation or manifesting lower vaginal recurrences in combination with lesions at two or more other pelvic sites; the survival rate in this stage is 23 %. It is of interest to note that the cases in stage II B categories with lower vaginal involvement have low survival rates.

The stage III level includes all cases with spread of cancer outside the pelvic region. The survival rate in this group is 15 %.

A method of assessing the effectiveness of the staging procedure is illustrated in Fig. 3. The survival rates of cases with the various stage levels were calculated at a time after the initial reference point (i.e. the time of clinical detection of recurrence in this study) when half of the total initial population had died.

Referring to the plotting of percentage cumulative stage incidence as the ordinate, and percentage survival determined at this time as the abscissa, the total sample population may be conceived as being distributed uniformly in the square area formed by the coordinates and separated by a diagonal between them into 'alive' and 'dead' patients. The alive patients would be distributed above the diagonal and the dead patients below. Due to the limits of sample size, a 49 % survival rate was the best possible calculable half population survival rate in this example.

The crux of the problem of staging then is the proper selection of definitions for each stage level so that cases so grouped have survival rates at each of the

Table 2

Results of radiotherapy in stage I recurrent endometrial carcinoma

Treatment	32 month survival
Rad um	20/24 (83)
Roentgen	5/8 (63)
Roentgen and rad um	13/15 (87)

Table 3

Results of radiotherapy in stage II recurrent endometrial carcinoma

Anatomic subdivisions	32 month survivals		
	Rad um	Roentgen	Roentgen and rad um
Lower part of vagina	2/4 (50)	2/4 (50)	2/3 (67)
Lesions with pelvic spread (not fixed)		6/16 (37)	6/13 (46)
Lesions with pelvic spread (fixed)		1/9 (11)	4/9 (45)
Total	2/4 (50)	9/29 (31)	12/25 (48)

levels parallel to and close to the diagonal. The closeness then to which the stage levels approximate the diagonal is a rough measure of the efficiency of the staging system evolved. Furthermore, each staging level should be distinctly separated from the others and encompass a good part of the diagonal. It should be noted that if survival rates for each of the stage levels are computed at a time point when the total sample population rate is greater than 50 % the plotted values will be shifted to the right of the diagonal. The diagonal will be curvilinear with a central sag toward the right hand corner dividing the area into alive and dead proportions and vice versa. Hence the use of total rates differing greatly from 50 % complicates this procedure and necessitates a formal expression of the cumulative incidence of stage levels and percentage survival values.

The cross or double cross plots indicate the cumulative incidence of stage against survival rate whereas the triangle or diamond plots give the midpoint stage interval of cumulative incidence against rate. The horizontal lines are the estimates of the 90 % confidence intervals. The vertical dotted lines divide the subsample population for each of the stage levels into alive and dead subjects.

All stages except stages II B and III are prognostically distinct ($p < 0.02$). Stages I, II A and III lie close to the diagonal but stage II B is somewhat

Table 4

Comparison of three staging systems in carcinoma of the corpus uteri

Anatomic sites	International (1963)	CHURCH (1966)	HIRABAYASHI (1968)
Corpus	I	—	—
Normal	—	I (< 9 cm)	—
Enlarged	—	II (9–10 cm)	I (< 8.5 cm)
Markedly enlarged	—	III (> 10 cm)	II (> 8.5 cm)
Cervix	II	—	III
Parametria	III	IV	IV
Pelvic wall	III	IV	IV
Distant	IV	—	—

deviant. The best prognostic divisions are stages I, II A, and II B plus III, but for treatment purposes it would be better to combine the cases with pelvic extensions (stages II A plus II B) or to keep stage II B as a separate category, as in Table 1. The latter system is also prognostically distinct when stages II A and II B are combined. However, it is not as good as the former, as demonstrated by the tendency of the survival rate of this combined group to overlap that of the stage III group.

Since survival (crude, tumor free or other types) is the known measure of the success of treatment procedures, it is necessary to equalize powerful prognostic factors, such as stage, among the treatment groups before the comparison. If this equalization is omitted, a heterogeneity of stage levels will almost inevitably lie between or among the treatment groups, which if not taken into account, must lead to a misinterpretation of the relative efficacy of the various treatment methods that are being compared. That such misinterpretation is all too common is readily evidenced by inspection of the current medical literature.

Tables 2 and 3 are presented to demonstrate the applicability of this or a similarly constructed staging system for the comparison of treatment methods. If it is accepted that duration and possible morbidity of roentgen therapy are undesirable compared to vaginal radium treatment, then the latter alone would appear to be the best modality for stage I lesions, whereas the need for external therapy is indicated when this neoplasm has extended into the pelvic regions. The controversy relative to the radiologic and surgical management in recurrent corpus carcinomas has been discussed elsewhere (BADIB *et al.* 1969).

Since other staging systems for recurrent carcinoma of the corpus uteri were not encountered in the literature, those previously established for primary cancers of this organ were utilized for the purpose of demonstration. The three

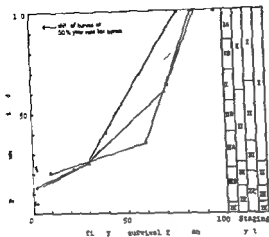


Fig 4 Assessment of staging systems in primary carcinoma of the corpus uteri Roswell Park Memorial Institute Series 1945-1961 345/563 (61%)

systems chosen for evaluation are presented in Table 4. Data from a recent publication comparing three systems of staging applied to a single series of cases (HIRABAYASHI et al 1968) have been employed.

A total of 136 out of 766 cases were excluded in the cited study prior to the calculation of survival rates because they had succumbed from other causes. Unfortunately, this removal of an unknown proportion of cases five or more years after the diagnosis tends to produce a bias toward lowering the survival rates more than when these rates are calculated on a time at risk basis and are then corrected for non-cancer deaths. It also might have been somewhat more desirable to use the percentage survival values at a time when half of the total population at risk had expired, i.e. some time after five years rather than those at five years. The first bias tends to shift the plotted values to the left while the second causes a shift to the right. For these reasons the survival rates required for this method of assessment cannot be ascertained from these data. However, this uncertainty naturally does not interfere with the use of these data for a demonstration of the method described in this report. As mentioned above, when a staging system approaches an ideal system, the plotted values of each level should be parallel to the diagonal (Fig 3) or to some curvilinear off-diagonal dividing line, distinctly separate from each other.

The international system (See Annual Report on the Results of Treatment of Carcinoma of the Uterus 1958) approaches proximity to the diagonal band at stage levels II, III, and IV, but the latter two are prognostically inseparable (Fig 4). All three measure only the lower third of the diagonal. Its stage I group includes disproportionately large portion of the patient population with

most of the survival information lost within it. It is the most deviant of the three GUSBERG's (1966) and HIRABAYASHI's (1968) systems tend to follow the downward curvilinear diagonal line dividing the total sample into 60% alive and 40% dead patients. However, in the former system the gap between stages III and IV is relatively large, whereas in the latter the gap between II and III is large. It would appear therefore that an opportunity exists to make further worthwhile improvements in the existing staging systems for primary corpus carcinoma. For instance, by including in a staging system the variables, uterine cavity depth and histologic grade, along with the currently used anatomic variables, the staging system thus evolved might more closely approach an ideal one, as assessed according to the scheme now proposed.

Another consideration involves the use of prognostic variables with continuous scores, e.g. cm diameter of lesion, cm depth of uterine cavity, years of age. These should ideally be demarcated into intervals by determining points at which the survival rates change, before incorporating them with the various anatomic components. For example, in primary cervix cancer without extension, tumor size in terms of small and large diameter should be demarcated by a point separating them into centimeter intervals predicting the greatest difference in rates possible.

Discussion

The primary concept of measurement in the clinical staging of cancer is the prediction of outcome of the disease. To develop this concept, knowledge of the manner of growth and of direction of spread of the cancer entity must be variable. Correlative clinicopathologic studies may be used to establish or indicate that the important clinical variables in defining staging systems for each histologic type of cancer are (1) the anatomic sites of involvement, (2) tumor size, (3) depth of invasion and (4) histologic grade. Other known variables seem less important but may be added to modify the system, e.g. age, symptoms, duration, treatment, concurrent disease as appropriate.

Although the prognostic significance is known individually for many of the clinical as well as of other types of variables, little is known about the degrees of their correlation or interdependencies. In a highly structured multivariate system, as in human beings, there are variables exhibiting a wide range of correlations, from complete independence to complete dependence. Well correlated variables contribute only redundant information on prognosis so that they should be combined into a few clusters.

Partially correlated variables manifest their influence in varying degrees through others, so that those with the strongest effects on prognosis should be

removed from those with less in decreasing order of effects until those with little or none are encountered. The latter should be eliminated. In this way it is possible greatly to reduce the numbers of variables affecting prognosis, permitting an easier handling of those remaining, and minimizing the danger of mixing those with different levels of prognostic distinctions.

There is danger in simplifying staging systems on the basis only of practicability or perhaps merely to incorporate large numbers of cases in each stage level selected. Such lumping together of unknown proportions and unidentified content of artificially defined staging variables may yield precise but useless generalities which may well be inaccurate and valueless when applied to individual patients. The stipulation made in connection with changes in the staging systems for various types of cancer in the past suggest that some such considerations may have induced these changes.

Even in the time honoured highly successful international classification of the stages of carcinoma of the uterine cervix certain discrepancies are evident. For example involvement of the bladder or rectum or both without reference to other sites cannot possibly correlate with other variables cited for inclusion of cases in the stage IV category. Cases of local cervical lesions with bladder or rectal involvement at the time of diagnosis are likely to do much better than those with bilateral pelvic wall fixation and such involvement. Treatment wise these cases should therefore also be placed in different categories. The former may more likely be amenable to pelvic exenteration procedures with relatively good results whereas the latter may not.

The separation and combination of objects for classification is sometimes referred to as lumping and splitting. The initial splitting is mandatory in clinical analyses without these initial divisions the clinician lumps a melange of unknown entities. Having split he can always lump later but he then has the major scientific advantage of knowing what he lumps and why (FEINSTEIN 1967) i.e. after he has knowledge of the distinctiveness of the objects.

Concepts or definitions for the measurement of prognostic variables vary for different types of cancer originating in different organ systems. Regional lymph nodal involvement cannot be assessed with the same degree of clinical validity in gynecologic cancers as in those of the head and neck. This is evident in the TNM system initially developed for the latter but recently proposed for cancers of the uterine cervix.

In contrast to head and neck cancer nearly all of the prognostic information in carcinoma of the cervix uteri is contained in the T categories and little or none in the N categories while M1 is entirely contained in stage IV B. Furthermore the Nx category has not been ordinarily sought for or discovered in cancer of the cervix in most clinical situations. It will be interesting to observe how well

most of the survival information lost within it. It is the most deviant of the three GUSBERG'S (1966) and HIRABAYASHI'S (1968) systems tend to follow the downward curvilinear diagonal line dividing the total sample into 60 % alive and 40 % dead patients. However, in the former system the gap between stages III and IV is relatively large, whereas in the latter the gap between II and III is large. It would appear therefore that an opportunity exists to make further worthwhile improvements in the existing staging systems for primary corpus carcinoma. For instance, by including in a staging system the variables, uterine cavity depth and histologic grade, along with the currently used anatomic variables, the staging system thus evolved might more closely approach an ideal one, as assessed according to the scheme now proposed.

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Although the prognostic significance is known individually for many of the clinical as well as of other types of variables, little is known about the degrees of their correlation or interdependencies. In a highly structured multivariate system, as in human beings, there are variables exhibiting a wide range of correlations, from complete independence to complete dependence. Well correlated variables contribute only redundant information on prognosis so that they should be combined into a few clusters.

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REFERENCES

- BADIS A O KUROHARA S S BEITIA A A and WEBSTER J H Recurrent cancer of the corpus uteri II Techniques and results of treatment *Amer J Roentgenol* 105 (1969) 596
- BASCO F and KUROHARA S S Combinatorial attribute analysis computer program To be published
- CLINICAL STAGING SYSTEMS FOR CARCINOMA OF THE CERVIX Joint Committee for Cancer Staging and End Results Reporting 1964
- FEINSTEIN A R Clinical judgment Williams and Wilkins Co Baltimore 1967
- GINSBERG S B The problem of staging endometrial cancer *Obstet and Gynec* 28 (1966) 305
- HIRabayashi K and GRAHAM J B Classification of carcinoma of the uterine body *Surg Gynec Obstet* 126 (1968) 75
- KOTTMEIER H L The classification and clinical staging of carcinoma of the uterus and vagina *J Internat Fed Gynec Obstet* 1 (1963) 83
- KUROHARA S S BADIS A O BEITIA A A and WEBSTER J H Recurrent cancer of the corpus uteri Clinical features and prognosis *Acta radiol Ther Phys Biol* 8 (1969) 373
- RESULTS OF TREATMENT OF CARCINOMA OF THE UTERUS Annual Report Vol 11 Edited by H L Kottmeier Norstedts Stockholm 1958
- SNYATH P H A New approaches to bacterial taxonomy Use of computers *Ann Rev Microbiol* 18 (1964) 335

this system of staging works out in the actual clinical setting where the credibility of the TNM system must be eventually determined

The method described is believed to provide a logical medical taxonomic type (SWEATH 1964) approach to cancer staging. It is further believed that if utilized, it could reduce the production of numerous incompatible staging schemes derived by trial and error, the latter have probably been developed due to the classification with prior systems that have proved of little utility in the clinical setting. The method proposed may well permit experienced clinicians to design creditable staging systems allowing for simplicity and practicability by the logical lumping of subsets of cases with similar prognostic as well as clinical distinctions.

Acknowledgement

The authors take this opportunity of thanking Bernard Hanes for his help in suggesting the mechanics that might be applicable. This study was supported in part by the USC Medical School General Research Fund and by Social Security Research Contract 467.

SUMMARY

An objective nonempirical approach to the construction of clinical cancer staging systems is described. The method has been utilized as a pilot project to develop a staging system for recurrent endometrial carcinoma following total hysterectomy in a series of 149 cases. The system so devised has been evaluated and found to be accurate in terms of prognosis and accordingly an effective means of comparing treatment methods.

ZUSAMMENFASSUNG

Ein objektives nicht empirisches System der Einteilung von Krebs in verschiedenen Stadien wird beschrieben. Versuchsweise wurde dieses System zur Einteilung von 149 Fällen von rezidivierendem Karzinom des Endometriums nach vorhergehender Resektion des Uterus angewandt. Die neugeschaffene Stadieneinteilung wurde kritisch überprüft und zeigte sich prognostisch als zuverlässig und nützlich im Vergleich verschiedener Behandlungsmethoden.

RÉSUMÉ

Les auteurs décrivent une méthode objective et non empirique pour établir un système clinique de classement de cancer par stades. Cette méthode a été utilisée comme projet pilote pour mettre au point un système de classement des récurrences de carcinomes de l'endomètre après hystérectomie totale sur 149 cas. Ce système a été mis à l'épreuve et considéré comme précis en ce qui concerne le pronostic et par conséquent considéré comme un moyen efficace de comparaison des méthodes de traitement.

Table 1
Experimental conditions and ^{90}Sr doses employed

Dose of ^{90}Sr in $\mu\text{Ci/g}$ body weight	Total number of mice investigated	Number of mice killed in groups of five at given intervals	Last day for sacrifice	Number of mice that died naturally
<i>Sacrificed mice</i>				
16	120	65	300	50
0.8	121	75	360	46
0.4	122	95	480	27
0.2	122**	103***	540	17
Control	95	91****	570	1
<i>Mice used for cell distribution analysis</i>				
0.8	90	20	60	0
0.4	60	50	300	0
0.2	20	20	60	0
Control	50	50	300	0

Of these animals five and two were lost during the experiment only three*** and four**** animals respectively were sacrificed in the last test group

The extent to which different doses of ^{90}Sr affect the functional relations between the thymus, spleen and bone marrow will also be considered

Material and Methods Four groups of CBA male mice 75 days old were treated intraperitoneally with different doses of $^{90}\text{Sr}(\text{NO}_3)$. A group of 95 untreated animals were used as controls. Five mice from each group were selected at random and sacrificed at intervals of 7, 14, 21 and 30 days after injection of ^{90}Sr and then at monthly intervals until all the surviving mice in each series had been used up.

The quantitative relation between cells in the bone marrow, spleen and thymus was studied by distribution analysis. One hundred mice were separated into three groups and then injected with 0.2, 0.4 and 0.8 $\mu\text{Ci/g}$ body weight respectively. Fifty mice were used as untreated controls. Five mice from each of these groups were killed after 7, 14 and 21 days and after 2 months. In addition five mice from the control groups and from the 0.4 μCi group were investigated at 3, 4, 5, 7, 9 and 10 months. Experimental conditions and the doses of ^{90}Sr employed are recorded in Table 1.

PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF RADIOSTRONTIUM IN MICE

Changes in the haematopoietic system

by

AGNAR NILSSON

The early and late effects of ^{90}Sr on the haematopoietic system have often been investigated (2, 3, 7, 8, 9, 10, 12, 15, 19, 21) but they are in many respects poorly understood since the time dependent distribution in the tissues and the biologic behaviour of ^{90}Sr are very complex. Most of the tissues are initially subjected to short irradiation, before the radiostrontium is almost completely confined to the skeleton. The marrow will be particularly exposed but the irradiation dose will differ considerably depending on size and shape of the bone and the rate of redistribution of the ^{90}Sr in the different parts of the skeleton. The extraskelatal parts of the haematopoietic system may on the other hand to some extent be directly, but more and more indirectly, influenced by the successively decreasing regeneration capability of the bone marrow after protracted irradiation.

Some of these aspects have earlier been investigated and reported on (16, 17). The present paper deals with the quantitative and qualitative as well as the time dependent effects of various doses of ^{90}Sr on the haematopoietic system.

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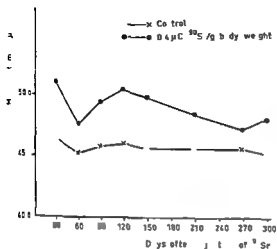


Fig 2 Mean volume of erythrocytes in peripheral blood in relation to time after injection of ^{90}Sr
Persistent macrocytosis

as possible the ^{90}Sr induced quantitative variations in the different cell populations. Parts of the thymus and spleen were suspended in homogenization tubes containing a relatively particle free balanced salt solution. After cutting both ends of the femur the marrow was forced out by compressed air and handled in the same way as the thymus and spleen. All the organ suspensions were adjusted to contain between 10^4 and 3×10^4 cells per milliliter. The peripheral blood was also investigated in suspensions of 1–2 000.

The cell volume distribution was studied with an electronic counter (Coulter Counter Model B) equipped with an automatic particle size distribution analyser (Coulter Plotter Model J). The principle and operation of this instrument have been described by BRECHER et coll (1962). The instrument was calibrated with rat erythrocytes and essentially monosized Ragweed Pollen for the absolute cell volume determination.

The cells investigated were divided into small cells of size 40 to $100 \mu^3$, moderate sized cells 100 to $200 \mu^3$ and large cells 200 to $500 \mu^3$. The limits for the three groups of cells were approximate but chosen as far as possible to represent the main bulk of morphologically defined cell entities, they seem to agree fairly well with the results obtained by HAOT et coll (1967). The reason for setting the upper limit for the small cells at $100 \mu^3$ was that the main part of these cells consists of erythrocytes with a mean volume of between 40 and $50 \mu^3$ (range 20 to $140 \mu^3$). The influence of leukocytes on the volume distribution of the peripheral blood could be designated as insignificant. The moderate sized cells were chosen within limits mainly representing the lymphoid cell elements. Their mean volume was between 120 and 140 with a range of 100

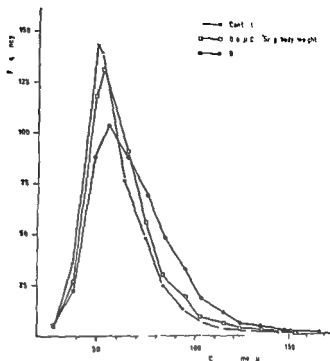


Fig. 1 Cell volume diagram (Coulter counter) of peripheral blood with macrocytosis 21 days after injection of ^{90}Sr

The mice before sacrifice were anesthetized and blood samples were obtained with a Pasteur pipette from the medial venous plexus of the eye. Total counts of leukocytes and thrombocytes were made. The haemoglobin values were expressed in gram per 100 ml blood. Blood smears for the differential counts were dried in air, stained with May Grunewald Giemsa, and 200 cells were counted in each smear.

For the histologic investigation, the femora, tibiae and humeri as well as the pelvic bones, the spine, thymus, spleen, mesenteric lymph nodes, liver, kidneys and the brain were fixed in Stieve's fluid. These tissues were prepared according to conventional histologic methods and stained by the van Gieson method, haematoxylin and eosin, Lillie's azure eosinate and PAS according to Hotchkiss.

The bone marrow in the distal, proximal and diaphysal parts of each pair of the long bones and in the cervical, thoracic, lumbar and sacral spines, as well as in the regions of the tuber sacrale, tuber ischii, ilia, acetabula and basis crani were analysed with respect to their degree of cellularity. The cellularity of the marrow was classified according to an arbitrary scale from 0 (aplastic marrow) to 5 (normal marrow). The cellularity for a group of five mice was expressed as the mean obtained from 155 measurements.

The analysis of the cell volume distribution was made in combination with the investigation of the histologic features in order to demonstrate as objectively

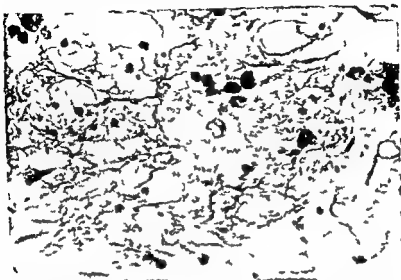


Fig 3 Haemosiderin loaded macrophages in aplastic and necrotic bone marrow of femur 300 days after injection of 16 μCi $^{90}\text{Sr/g}$ body weight (van Gieson \times 500)

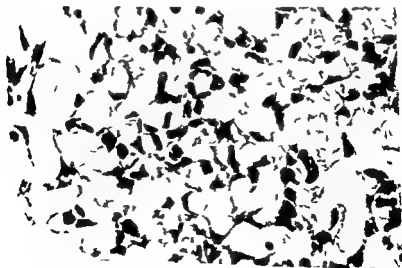


Fig 4 Proliferation of reticular cells in aplastic bone marrow of diaphysis of femur 300 days after injection of 0.8 μCi $^{90}\text{Sr/g}$ body weight

to 200 μ^3 . The 'large cells' were mostly various types of blasts (usually over 200 μ^3)

Results

Peripheral blood A significant and persistent leukopenia developed in all the dose groups at 7 days. Expressed as the mean for the whole experimental period the following dose related levels of leukocytes were observed

1 687.7 \pm 153.2 (range 1 072—2 970) in the 1.6 μCi group

3 063.7 \pm 181.4 (range 2 112—4 548) in the 0.8 μCi group

4 223.5 \pm 213.3 (range 2 980—5 972) in the 0.4 μCi group

4 837.9 \pm 238.2 (range 2 820—6 948) in the 0.2 μCi group

8 383.3 \pm 247.8 (range 6 836—10 378) in the control group

A shift in the differential counts also occurred. The mean percentage distribution of granulocytes in the ^{90}Sr treated groups was over 30 % (range 25.6—38.4 in the 1.6 μCi group, 29.0—62.8 in the 0.8 μCi group, 23.6—43.8 in the 0.4 μCi group and 25.6—36.0 in the 0.2 μCi group). The control value was 19.9 % (range 15.6—29.2). A corresponding and persistent lymphopenia was observed.

The hemoglobin values in all the dose groups reached a minimum within 14 days. A slight recovery then occurred but normal values were never regained in any one of the groups. The mean values (g/100 ml blood) for the whole experimental period were as follows

11.2 \pm 1.95 (range 9.9—12.5) in the 1.6 μCi group

11.7 \pm 1.52 (range 10.4—12.4) in the 0.8 μCi group

12.1 \pm 1.70 (range 10.7—13.4) in the 0.4 μCi group

12.5 \pm 1.02 (range 11.4—13.2) in the 0.2 μCi group

13.3 \pm 2.14 (range 11.6—15.6) in the control group

Marked macrocytosis (Fig 1) accompanied by an increase of polychromatic erythrocytes were present in the peripheral blood in all the dose groups investigated (0.2, 0.4, 0.8 μCi ^{90}Sr /g body weight) as early as at 7 days. The macrocytosis persisted when investigated for a period of up to 300 days after the injection of 0.4 μCi ^{90}Sr /g body weight (Fig 2).

The thrombocyte counts reached their minimum values at 14 days in all the dose groups but thereafter a rapid increase to normal or even higher values occurred. The following mean values were obtained

532 000 \pm 51 900 (range 247 800—771 000) in the 1.6 μCi group

536 000 \pm 50 500 (range 225 600—854 800) in the 0.8 μCi group

549 500 \pm 39 400 (range 196 000—984 000) in the 0.4 μCi group

596 100 \pm 40 800 (range 259 600—885 000) in the 0.2 μCi group

531 000 \pm 19 000 (range 384 400—720 750) in the control group



Fig 3 Haemosiderin loaded macrophages in aplastic and necrotic bone marrow of femur 300 days after injection of 16 μ Ci ^{90}Sr /g body weight van Gieson $\times 500$

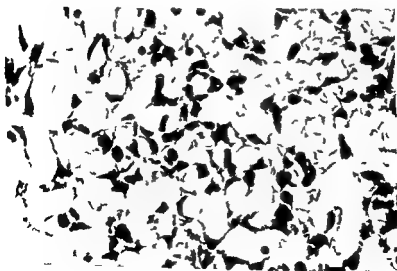


Fig 4 Proliferation of reticular cells in aplastic bone marrow of diaphysis of femur 300 days after injection of 0.8 μ Ci ^{90}Sr /g body weight

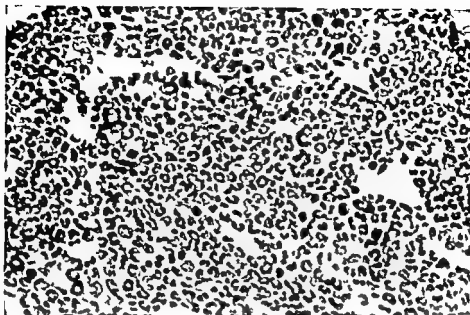


Fig. 5. Bone marrow of femur. Granulocyte proliferation 150 days after injection of $0.2 \mu\text{Ci } ^{90}\text{Sr}/\text{g body weight}$ van Gieson $\times 500$.

Bone marrow. The histologic changes differed quantitatively but were principally of the same type in all the dose groups. Dilatation and hyperaemia of the medullary sinusoids accentuated with increasing dose. Increased amounts of fatty tissue appeared, more abundant and earlier in the two higher dose groups than in the others. Appreciable amounts of fat appeared in the $1.6 \mu\text{Ci}$ group as early as at 7 days.

Destruction of the sinusoids with formation of localized blood lakes was more extensive in the lower than in the higher dose groups. This was the case particularly at 270 days in the $0.4 \mu\text{Ci}$ group. Thromboses in different stages of organisation were also a common feature long after the injection of ^{90}Sr , particularly in the $0.2 \mu\text{Ci}$ group at 390 days but also in the $0.4 \mu\text{Ci}$ group. They appeared most frequently in the diaphysal part of the marrow of the long bones.

Marrow necrosis was present, above all in the femur, in all the dose groups, but earlier (Fig. 3) in the $1.6 \mu\text{Ci}$ group (210 days) and the $0.8 \mu\text{Ci}$ group (360 days) than in the others. In many aplastic or markedly hypoplastic marrows, only reticular cells remained, or were increased, or were in a state of proliferation (Fig. 4). This occurred particularly in mice given $0.4 \mu\text{Ci/g}$ body weight. These cells have in earlier work (SUNDELIN & NILSSON 1968) been shown to start the development of predominantly fibroblastic osteosarcomas.

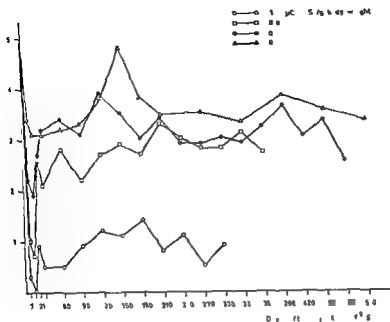


Table 2

Distribution of mice (per cent) in relation to ^{90}Sr dose and bone marrow cellularity

Dose group $\mu\text{Ci/g}$	Bone marrow cellularity					
	Aplastic 0.0—0.5	Markedly hypoplastic 0.6—2.5	Moderately hypoplastic 2.6—3.5	Slightly hypoplastic 3.6—4.5	Normal 4.6—5.0	Hyperplastic >5
1.6 n 65	32.3	67.7	0.0	0.0	0.0	0.0
0.8 n 75	0.0	45.4	52.0	0.0	2.7	0.0
0.4 n 95	0.0	19.0	68.4	8.4	3.2	1.1
0.2 n 103	0.0	0.0	60.0	32.9	5.7	1.4

in regeneration during a short period (before 30 days). The granulocytic series started to dominate at 90 days in the 0.8 μCi group and after about 120 to 150 days in the others. This granulocytosis was temporary in the 0.2 μCi group and ended after about 360 days.

Marked hyperplasia, particularly in the bone marrow but also in the spleen, of hardly any other cells than those representing the granulocytic series, occurred in several instances, especially in the lower dose groups (Fig. 5).

Megakaryocytes were more abundant than normally in the 0.4 and 0.2 μCi groups, and this particularly between 330 and 390 days. In the 1.6 μCi group, megakaryocytes reappeared after the initial period but never attained their normal number.

An abundance of mastocytes were seen in some instances in the 0.8 and 0.4 μCi series in the bone marrows and in most lymphoid tissues, including the thymus.

Quantitative analysis. The effect of the various doses of ^{90}Sr on the bone marrow cellularity among the sacrificed mice is recorded in Fig. 6. The minimum values were observed in all series at 14 days, the cellular depletion increasing with increasing dose. The marrow depletion within each group was initially of about the same magnitude in the various marrow cavities investigated but the start and degree of marrow regeneration differed greatly in the various parts.

The percentage distribution of sacrificed mice in relation to dose and their mean bone marrow cellularity is shown in Table 2. Six out of fifty mice in the

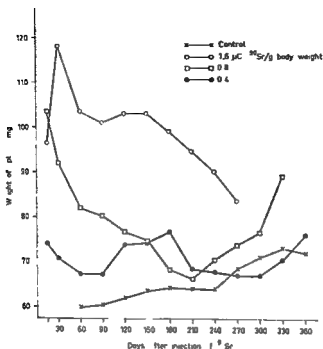


Fig 7 Weight of spleen in relation to dose and time after injection of ^{90}Sr

1.6 μCi group died naturally of acute and four of chronic aplastic anaemia. Chronic aplastic anaemia occurred in the 0.8 μCi group in one out of forty six and in the 0.2 μCi group in one out of seventeen mice.

The Coulter analysis revealed a prominent and persistent increase of small cells mostly representing the engorgement of the sinusoidal space by erythrocytic elements. The degree of hyperaemia seemed to be initially related to dose, i.e. the degree of hyperaemia increased with increasing dose. Moderate sized cells representing lymphoid elements were considerably reduced and never regained normal values nor did the large cells representing blasts.

Marrow regeneration Topographically the start and intensity of regeneration varied and proceeded according to the bone in which the marrow was located. The marrow of the femurs in the 1.6 μCi group presented little evidence of regeneration apart from scattered small foci of dividing cells and was practically aplastic until the death of the animals. On the other hand considerable regeneration took place focally in the marrows of the tuber sacralae tuber

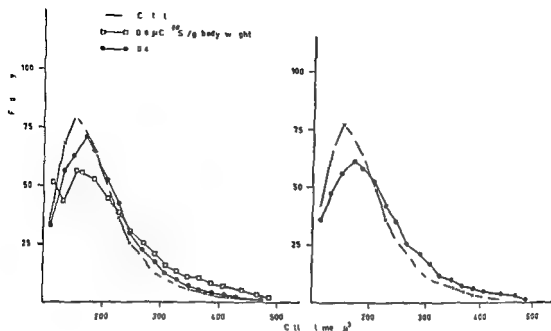


Fig 8 Cell volume diagram (Coulter counter) for spleen 21 days (left) and ten months (right) after injection of ^{90}Sr . Dose related and persistent lymphocyte depletion

ischii, the thoracic vertebrae as well as in the sternal marrow, and reached its peak at about 180 days after the injection of the ^{90}Sr . Cells of the granulocytic series predominated in these islands of regeneration.

Also in the other groups the marrow of the femur was more damaged than other parts. The most active marrows were present at the same sites as in the $1.6 \mu\text{Ci}$ group but the regenerative capacity was greater and related to dose, reaching its peak in the $0.8 \mu\text{Ci}$ group at 210 days, as compared to between 120 and 150 days in the other groups. The decreased marrow activity in the femurs was however compensated for predominantly in the thoracic vertebrae and sternal marrow.

Spleen Changes in the weight of the spleen are illustrated in Fig 7. In spite of a heavy loss of lymphatic tissue in the higher dose groups and a moderate loss in the two lower dose groups (Fig 8), a most significant increase in weight persisted over a long period, this was caused by hyperaemia and a heavy erythro-, megakaryocyto- and granulocytopoiesis (Fig 9). The degree of lymphoid depletion depended upon the dose.

Histologically the findings largely agreed with those described in an earlier paper (16). It should however be noted that in the $1.6 \mu\text{Ci}$ group the increased extramedullary blood formation, which was initially dominated by the erythroid

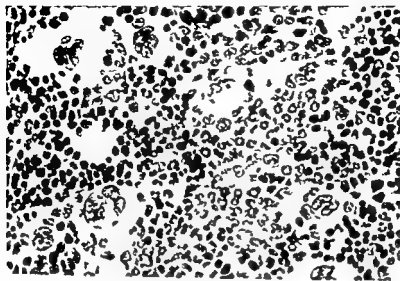


Fig 9 Appearance of cells of spleen two months after injection of 1.6 μCi $^{90}\text{Sr/g}$ body weight. Extra medullary haematopoiesis of all types of cells. H-E $\times 500$.

series was successively and after about 200 days completely replaced by the granulocytic cell series.

Extramedullary haematopoiesis in the 0.8 μCi group was marked but in contrast to the 1.6 μCi group erythroid elements were usually more abundant in the process of regeneration than the granulocytic series of cells.

Megakaryocytopoiesis was much more prominent in the two lower dose groups than in the two others.

Thymus Changes in the weight of the thymus in the control groups and in the different ^{90}Sr series are illustrated in Fig 10. The loss of weight and enhanced rate of involution are dose dependent and as shown by the cell volume distribution analysis predominantly caused by a depletion of lymphoid cells (Fig 11). This lymphoid depletion was not detectable histologically.

Discussion

A general depletion of all haematopoietic tissues occurred in all the dose groups but only in the 1.6 μCi group was a high frequency of aplasia recorded among the sacrificed animals (see Table 2). In this group the mice also died naturally from acute aplastic anaemia.

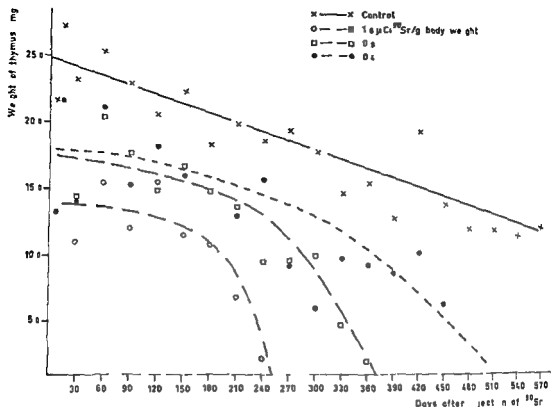


Fig 10 Weight of thymus in relation to dose and time after injection of ^{90}Sr

The weight of the thymus diminished increasingly with enhanced dose and the weight of the spleen increased progressively. It is obvious from the cell volume distribution diagram that the diminished weight of the thymus is caused by a constant depletion of lymphocytes, predominantly in the cortical zone. Persistent lymphoid depletion occurred also in the spleen but the increased weight was brought about by hyperaemia and an extramedullary diffuse haematopoiesis, the intensity of which mainly mirrored the degree of damage to the bone marrow, in which practically all cell types were depressed, and the degree of this depletion was largely related to dose.

The destruction of the sinusoids in the bone marrow may have been directly related to the effect of irradiation since it was most widespread at the highest dose level. Vascular disorders may have been indirectly involved, as was indicated by the more frequent occurrence of blood lakes and necrosis in conjunction with thrombosis in the lower dose groups. It should be noted in this connection that thrombocytosis predominated, particularly in the lower dose groups.

In the regeneration of bone marrow a general pattern could be distinguished

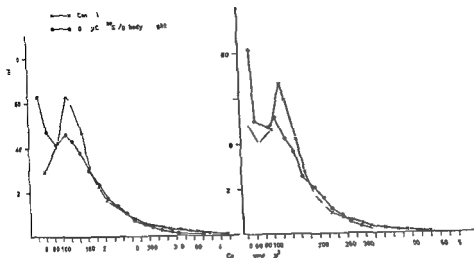


Fig 11 Cell volume diagram (Coulter counter) for thymus one month (left) and ten months (right) after injection of ^{90}Sr . Persistent lymphocyte depletion.

irrespective of the dose. Topographically this regeneration was mainly determined by the redistribution and amount of ^{90}Sr released and by the size and shape of the bone. The most active locations were the thoracic vertebrae, the sternum and certain sites in the pelvic bones. The evidence of regeneration was significant in the $1.6 \mu\text{Ci}$ group with the exception of sites in which cells of the granulocytic series generally predominated. In the other dose groups the regeneration with decreasing dose was more diffusely distributed. A persistent predominance of the granulocytic over the erythroid series in the marrow also occurred but started somewhat later with decreasing dose. This granulocytosis was intense but temporary in the $0.2 \mu\text{Ci}$ series and decreased or disappeared after about 360 days.

The bone marrow seemed to play a comparatively small role in the thrombocytopoiesis. Even though most of the marrow was destroyed in the highest dose group no peripheral thrombocytopenia developed since sufficient compensatory megakaryocytopoiesis took place in the spleen.

A generally diminishing erythropoiesis in the marrow occurred with increasing dose. This was however principally taken over by the spleen but was largely of insufficient magnitude as apparent from an increased macrocytosis and a diminished haemoglobin content. At the highest dose level and with progressing time this capacity of the spleen gradually decreased in favour of a more accentuated granulocytopoiesis. None of the destructive effects observed

in lymphocytopoiesis could be fully compensated for, and there was as a consequence a discernible shift in the relative proportions of granulocytes and lymphocytes in the peripheral blood.

The dose related, persistent and absolute decrease in granulocytes in the peripheral blood principally mirrors the diminished granulocytopoietic capacity, particularly of the bone marrow. It is however noteworthy that especially in the two lowest dose groups the granulocytosis, accompanied by a proliferation of myeloid elements, was sometimes of such extent and intensity that it might be identical with the leukemoid reaction observed in rats by BOEGLER & KRIEGER (1968), and the so-called myeloproliferative disorders in beagles (GOLDMAN *et al.* 1969), characterized by a terminal increase and a spectrum of disorders varying from myeloid metaplasia to granulocytic leukaemia. One of the causes of this reaction may be related to the fact that the granulocytic cell series is more radiation resistant than the erythroid and lymphoid series (1-3).

As regards the mutual relationship between thymus, bone marrow and spleen it is obvious that the involution of the thymus is accelerated and accentuated by an increasing dose, which may be explained on the basis of a dose related scarcity of bone marrow stem cells capable of repopulating the thymus. A generally enhanced consumption of thymus lymphocytes with or without destruction by the continuous irradiation from the bones surrounding the thymus may also be factors to consider. The spleen seems at least in the higher dose groups to serve as a reserve to compensate for the diminished bone marrow function. This interrelationship seems to be of some importance for a better understanding of differences in the leukemogenesis between ^{90}Sr and fractionated external irradiation. Most cases of leukaemia induced by the latter method are of thymic origin (cf. JARPLID 1968) in contrast to those induced by ^{90}Sr (8, 13, 18). With fractionated irradiation the entire haematopoietic system is repeatedly and uniformly damaged, and stem cells capable of repopulating the thymus may be acutely lacking (cf. JARPLID). The bone marrow is continuously irradiated with ^{90}Sr but the degree of damage and regeneration varies considerably in different parts of the marrow, while other parts of the haematopoietic system initially are not damaged to the same extent as by external irradiation.

SUMMARY

The quantitative and qualitative effects of various doses of ^{90}Sr on the haematopoietic system in four groups of CBA male mice and in controls are described.

ZUSAMMENFASSUNG

Der quantitative und qualitative Effekt von verschiedenen Dosen von ^{90}Sr an vier Gruppen von männlichen CBA Mäusen und an Kontrolltieren werden beschrieben

RÉSUMÉ

L'auteur décrit les effets quantitatifs et qualitatifs de diverses doses de ^{90}Sr sur le système hématopoïétique dans quatre groupes de souris CBA mâles ainsi que sur des témoins

REFERENCES

- 1 BAUER R. Untersuchungen über die Einwirkung unterschiedlich verabfolgter Röntgenstrahlung auf das Knochenmark und seine Zellelemente so gleich ein Beitrag zum Zeitfaktorproblem. *Strahlentherapie* 67 (1940) 424
- 2 BELOBORODOVA N L, BARANOVA E F. Investigation of the functional state of the blood formation system in rabbits subjected to continuous administration of radioactive cesium strontium and ruthenium. U S Atomic Energy Commission Translation Series AEC-tr 3794 (1957) 195
- 3 BLOOM M A. Bone marrow. In: *Histopathology of irradiation from external and internal sources* p 167. Edited by W. Bloom. McGraw Hill Book Company New York Toronto London 1948
- 4 BOZGLER F und KRIEGLER H. Leukämische Reaktion nach ^{90}Sr -Strontium Inkorporation bei Ratten. *Blut* 17 (1968) 345
- 5 — — Strahlenspatschaden nach ^{90}Sr Strontium Inkorporation bei Ratten. Gemeinsame Jahrestagung 1968. Deutsche Gesellschaft für Biophysik u v Österreichische Gesellschaft für reine und angewandte Biophysik. Baden bei Wien. September 1968
- 6 BRECHER G, JAKOBEK E F, SCHWEIDERMAN M A et coll. Size distribution of erythrocytes. *Ann N Y Acad Sci* 99 (1962) 242
- 7 BURYKINA L N. Changes in the peripheral blood following a high dose administration of radioactive ruthenium strontium and cesium. U S Atomic Energy Commission Translation Series AEC-tr 3794 (1957) 69
- 8 COSGROVE G E and UPTON A C. Some late effects of radiostrontium in RF male mice. *J nucl Med* 3 (1962) 293
- 9 EDINGTON G M, WARD A H, JUDD J M and MOLE R H. The acute lethal effects in monkeys of radiostrontium. *J Path Bact* 71 (1956) 277
- 10 GOLDMAN M, DLUGOWORTH D D, ROSENBLATT L S and BUSTAD L K. Radiation induced neoplasms in beagles following administration of ^{90}Sr and ^{224}Ra . Symposium on radiation induced cancer. SM 118. Athens 1969
- 11 HAOT J, RÉVÉZ L et HAUGHTON G. Une nouvelle méthode d'étude de la moelle hématopoïétique basée sur la détermination des volumes cellulaires. *Bull Cancer* 54 (1967) 159
- 12 ITO T, YOKORO K, ITO A and NISHIHARA E. A comparative study of the leukemogenic effects of strontium 90 and X rays in mice (33 553). *Proc Soc exp Biol Med* 130 (1969) 345
- 13 IVANOVA T A. The specific features of haematopoiesis in rats and their offspring in the action of Sr^{90} . *Med Radiol* 5 (1960) 41

- 14 JARPLID B Radiation induced asymmetry and lymphoma of thymus in mice *Acta radiol* (1968) Suppl No 279
- 15 MELCHNER H Veränderungen des weissen Blutbildes bei chronischer Strahlenbelastung durch Sr^{90} Inkorporation bei der Albinoratte *Naturwissenschaften* 46 (1959) 567
- 16 NILSSON A Effects of radiostrontium on the blood and hematopoietic tissues of mice *Acta vet scand* 3 (1962), 103
- 17 — Influence of gestation and lactation on radiostrontium induced malignancies in mice I Incidence distribution and characteristics of ^{90}Sr induced malignancies *Acta radiol Ther Phys Biol* 6 (1967), 33
- 18 — Pathologic effect of different doses of radiostrontium in mice Development and incidence of leukemia To be published in *Acta radiol Ther Phys Biol* 10 (1971)
- 19 PETROVICH I K Relationship between the cell composition of the sternal marrow and the peripheral blood in dogs in the acute phase of radiation sickness caused by strontium 90 *Probl Hemat Blood Transfus* 4 (1959) 34
- 20 SUNDELIN P and NILSSON A Cytoplasmic ultraviolet extinction of strontium 90-induced fibroblastic osteosarcomas correlated to histologic appearance and ultrastructure *Acta radiol Ther Phys Biol* 7 (1968) 161
- 21 YOKORO K Morbid anatomical and hematological studies on the effects of internal irradiation with Sr^{90} in mice (Japanese text) *Acta hemat jap* 21 (1958) 817 Cited from *Excerpta Medica (Radiology)* 13 (1959), 426, ref 1922

ELECTRON BEAM DEPTH DOSE DISTRIBUTION EVALUATION

by

A JACOBSON TH E BANKS M A ACKERMAN H E BRIZEL and R M SCOTT

The betatron beam therapy program was initiated at the Radiation Center of the University of Louisville when it was opened in 1964. Since that time our dosimetry rationale has placed more reliance on actual measured percent depth dose data than with precise beam energy determinations used in conjunction with published depth dose data. SCHULZ (1967) has advanced this viewpoint at the recent conference on high energy radiation therapy dosimetry in New York.

Toward this end we have pursued film dosimetry as our principal source of information. The known problems of film dosimetry were acknowledged and accepted in the absence of more feasible dosimetry techniques. Part of these problems were the tedious and possibly inaccurate manual densitometry of the films. There have been several successful approaches to reducing these objections (e.g. pantographic procedures and use of computers). We are using a commercially available film dosimetry system that is an improved version of the prototype developed by BOGARDUS et al. (1965).

Before giving a brief description of this system the film phantom arrangement will be discussed. The loaded phantom positioned for an exposure parallel to the

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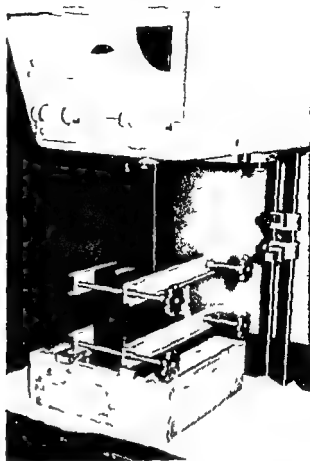


Fig 1 Phantom set up for betatron electron beam exposures. Ready pack industrial film is sandwiched in unit density rubber

electron beam is shown in Fig 1. The phantom consists of ready pack industrial film sandwiched between unit density rubber (Temex) slabs, which are in turn sandwiched in polystyrene slabs. Temex rubber was developed in England, as described by STACY et coll (1961). Its density is slightly less than that of polystyrene ($\text{Temex} \approx 1.02$ versus polystyrene ≈ 1.05). The principal virtue of this approximately unit density rubber is that it forms intimate contact with the test film. This is an important consideration in high energy electron film dosimetry because air occlusions result in differential electron scatter, with consequent greater local darkening of the film.

Use of light tight, pre wrapped industrial film avoids the clumsiness of loading the phantom with bare film in a darkened room. In addition to this simplification, we feel there is considerable benefit from more accurately loaded film phantoms than are possible when working in a darkened room. For example, in those circumstances where dosimetry is of interest in the electron build up region near the phantom surface it is imperative that the test film be properly located at that margin and clamped securely in the phantom. We have found

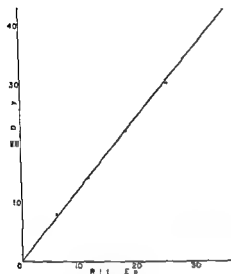


Fig 2 Characteristic curve for industrial type M film exposed to 24 MeV electrons

that the ready pack film gives results in this phantom similar to that of bare film. The experimentally determined characteristic curve for the industrial film used in our dosimetry is presented in Fig 2. This particular data is for 24 MeV electrons, and we have obtained linear curves for 14 MeV electrons also.

Industrial film exposure sensitivity is actually advantageous in this work since there is lesser involvement with background densities. The excellent detail property of this type of film makes it ideal for quantification in the densitometry system we use.

The film dosimetry system employed for the work described in this paper is manufactured by the Artronix Instrument Company, St. Louis. In Fig 3 is

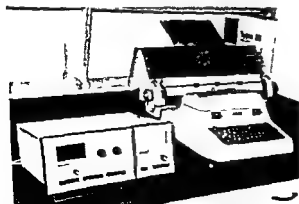


Fig 3 Film dosimetry system consisting of output writer (with test film) densitometer and printing percent digital voltmeter.

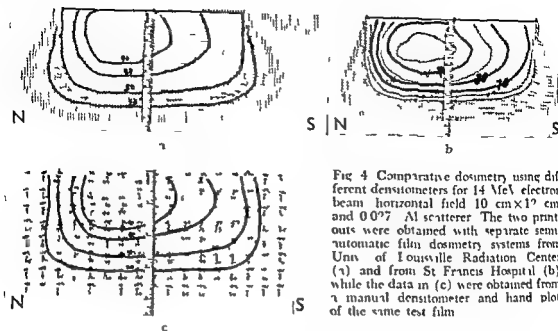


Fig 4 Comparative dosimetry using different densitometers for 14 MeV electron beam horizontal field $10 \text{ cm} \times 10 \text{ cm}$ and 0.077 Al scatterer. The two print outs were obtained with separate semi-automatic film dosimetry systems from Univ. of Louisville Radiation Center (a) and from St Francis Hospital (b) while the data in (c) were obtained from a manual densitometer and hand plot of the same test film.

shown a test film positioned in the output writer carriage containing a photocell assembly. The film is moved past the photocell in a predetermined pattern. Thus, the typewriter provides mechanical scanning, as well as a digital print out in 5% gradations. The variable density of the film would result in a variable output current of the PMT. However in this system the output current is maintained constant by a feedback method which changes the applied voltage to the PMT thereby providing a logarithmic output to correspond to the film density function. This output signal is processed and observed on the densitometer optical density scale. A portion of this signal is fed to the input of the printing percent digital voltmeter, where digital display is available.

The densitometer range is from zero to four density units and there are three range scales on the densitometer. The maximum density of the test film is located by manual movement of the output writer containing the film and simultaneous observation of the optical density on the densitometer. It is important to carefully select the reference point, and the zero point as well. We have substituted vernier potentiometers to facilitate selection of these cross over points. Periodic checks on the densitometer are made with a calibration wedge, and overall performance of the system is monitored by re-measuring of test films of known precision.

Comparative data print outs from three film dosimetry units are shown in Fig 4. A 14 MeV electron beam film was densitometered on our Artronic unit, on another such unit at St. Francis Hospital in Indianapolis, Indiana, and manually densitometered on our Welch densitometer. Qualitative and quantitative

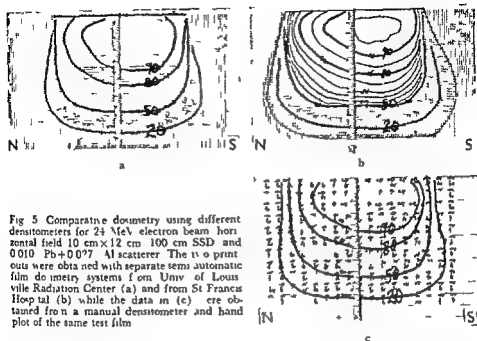


Fig 5 Comparative dosimetry using different densitometers for 24 MeV electron beam horizontal field $10\text{ cm} \times 12\text{ cm}$ 100 cm SSD and 0.010 Pb + 0.027 Al scatterer. The two printouts were obtained with separate semi automatic film dosimetry systems from Univ. of Louisville Radiation Center (a) and from St Francis Hospital (b) while the data in (c) are obtained from a manual densitometer and hand plot of the same test film.

agreement is quite apparent. Equally good comparative data was obtained for a 24 MeV electron beam film dosimetered on the same three systems (Fig 5).

Our semi automatic film dosimetry system was used to select the optimum beam flattening material for our electron beam. For clinical use it is desired to have the electron beam symmetrical in all planes properly flattened with minimal bremsstrahlung contamination and without excessive loss of beam output. Toward this objective lead aluminum and lead aluminum combinations have been used. Their location relative to the emergent electron beam is also critical because of their effect on resultant dose distribution. We have examined four scatterer arrangements available to us on our machine: 0.027 Al only, 0.010' Pb only, 0.010 Pb + 0.027 Al and 0.020 Pb only. All of these scatterers are positioned less than one centimeter from the exit window of the donut and ahead of the parallel plate ionization chamber monitor.

The field distributions in the plane perpendicular to the electron beam for all four of these scatterers at 24 MeV are presented in Fig 6. These distributions are in a plane 4 cm below the unit density rubber surface. It is seen that only the lightly scattered arrangement (0.027 Al only) fails to provide adequate flattening at this energy. Incidentally we have found that film dosimetry in the

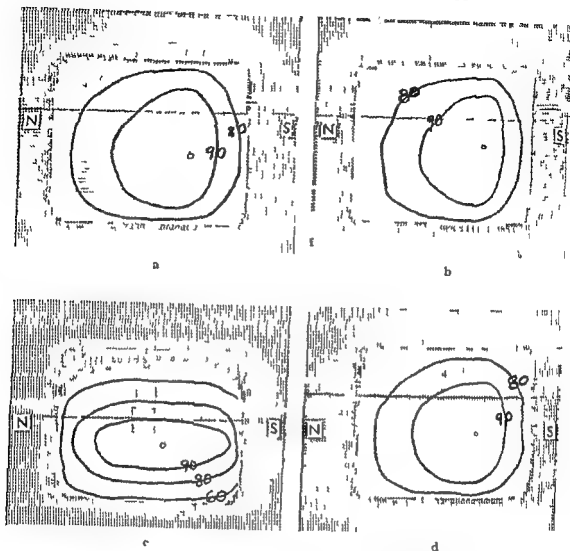


Fig 6 Cross sectional depth dose data (4 cm depth) for four different beam scatterer arrangements and 24 MeV electron beams 10 cm \times 12 cm fields 100 cm SSD 0.027" Al only (a) 0.010" Pb only (b) 0.010" Pb + 0.027" Al (c) and 0.020" Pb only (d)

plane shown in this figure, is invaluable in treatment planning. For example, the 80% depth dose line can be clearly visualized in another plane so the clinician may be assured that the tumor mass will be within this isodose boundary. The next three diagrams (Fig 7) show central axis dose distributions perpendicular to the plane of the donut (we routinely take phantom films in the plane parallel to the donut also). It will be seen that practically no bremsstrahlung is evident for the 0.027" Al only scatterer. But as we have just seen, insufficient beam flattening is provided at 24 MeV. The 0.010" Pb only and 0.010" Pb + 0.027" Al scatterers resulted in similar amounts of bremsstrahlung as the 0.027" Al only

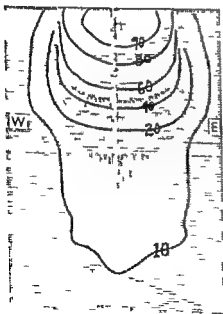
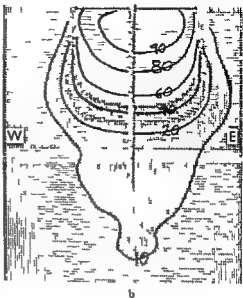
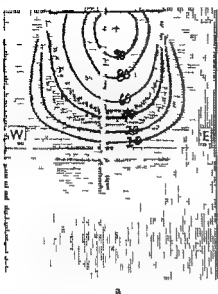


Fig 7 Depth dose data of three different beam scatterer arrangements 0.007 Al only (a) 0.010 Pb+0.007 Al (b) and 0.010 Pb only (c) These data were obtained from test films exposed in the plane parallel to the betatron donut

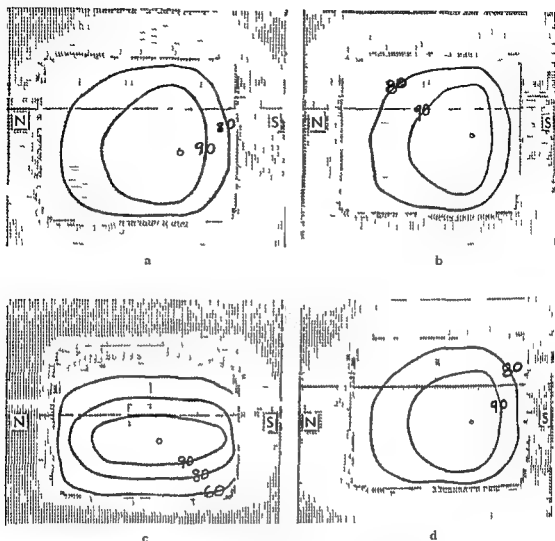


Fig 6 Cross sectional depth dose data (\pm cm depth) for four different beam scatterer arrangements and 24 MeV electron beams 10 cm \times 12 cm fields 100 cm SSD 0.027" Al only (a) 0.010" Pb only (b) 0.010" Pb+0.027" Al (c) and 0.027" Al only (d)

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response per rad for electrons and photons was the same within the experimental error of a few percent in the energy range 1—16 MeV

The comparative beam outputs for the four scatterer arrangements are recorded in the Table. Sec./Click and R/Click refer to beam monitor counts or 'clicks'. In our routine calibration method the beam monitor counts are calibrated against a Victoreen ionization chamber in an equilibrium block. Thus for the 14 MeV electrons scattered by 0.010' Pb + 0.027" Al each beam monitor click represents 6.2 R, and the frequency of these clicks gives an estimate of the exposure. It will be seen that our selection of the single scatterer (0.010' Pb + 0.027' Al) is made at the cost of considerable exposure. However the avoidance of technician error in improper scatterer selection compensates for this.

Subsequent donut replacements may necessitate another electron beam flattening method. With the system described here this determination will be simplified greatly over the previous methods available to us.

SUMMARY

A recently improved commercially available film densitometry system enabling convenient selection of depth dose distributions is described. Suitably obtained radiographic films of test exposures in unit density phantoms are densitometered and automatic digital display of the scanned film is produced in about 15 minutes. Examples are shown in which the undesirable bremsstrahlung component of the electron beam is quantified sufficiently for selection of optimum beam flattening arrangements.

ZUSAMMENFASSUNG

Ein kürzlich verbessertes kommerziell erhältliches Film Densitometer System, das eine bequeme Selektion der Tiefen Dosis Verteilung ermöglicht, wird beschrieben. Geeignete exponierte Filme von Test Bestrahlungen in Phantomen von Standard Dichte werden im Densitometer gelesen und eine automatisch digitale Darstellung des untersuchten Films kann in etwa 15 Minuten erhalten werden. Es werden Beispiele gegeben, bei denen die unerwünschte Bremsstrahlungs-Komponente des Elektronenstrahls quantitativ genügend erfasst wird, um eine Selektion optimaler Strahlen Abflachung zu ermöglichen.

RÉSUMÉ

Les auteurs décrivent un système de densitométrie par film récemment perfectionné et commercialisé qui permet une sélection convenable de distribution de dose en profondeur. Des films convenablement radiographiés soumis à des expositions de test dans des fantômes de densité unitaire ont été lus au densitomètre. On obtient en 15 minutes environ une représentation digitale automatique du film exploré. Les auteurs donnent des exemples dans lesquels la composante de bremsstrahlung indésirable du faisceau d'électrons est suffisamment quantifiée pour qu'on puisse choisir les arrangements qui donnent le meilleur aplatissement du faisceau.



Fig 8 Depth dose data with 14 MeV electron beam using 0.010 Pb only scatterer (a) and 0.010 Pb+0.027 Al scatterer (b) Increased bremsstrahlung component below 20 % dose for the 0.010 Pb only scatterer

scatterer The 0.020" Pb only scatterer shows an excessive amount, as seen from Fig 7c, the 20 % depth dose value is at about 11 cm depth, as compared with 10 cm depth for the 0.027" Al only scatterer The considerably deeper 10 % depth dose line for the former is also quite apparent The choice is now narrowed down to either the 0.010" Pb + 0.027" Al scatterer or the 0.010" Pb only scatterer

Fig 8 shows our basis for selection of the combination scatterer These distributions are taken at 14 MeV and it is seen that there is some additional bremsstrahlung present with the 0.010" Pb only scatterer

Comparative quantification of the bremsstrahlung component in the high energy electron film introduces no serious error DUDLEY (1966) has discussed this question in the new edition of Radiation dosimetry It was found that

Table

Comparison of electron beam outputs for various scatterer arrangements using a field size of 10 cm x 12 cm and 100 cm SSD

	Scatterer	Sec /Click	R /Click
14 MeV	0.027 Al	~2.0	12.4
	0.010 Pb	~4.5	6.3
	0.010 Pb+0.027 Al	~4.7	6.2
	0.020 Pb	~5.3	5.7
24 MeV	0.027 Al	~4.9	14.0
	0.010 Pb	~6.0	7.6
	0.010 Pb+0.027 Al	~6.9	7.6
	0.020 Pb	~8.9	7.0

response per rad for electrons and photons was the same within the experimental error of a few percent in the energy range 1—16 MeV

The comparative beam outputs for the four scatterer arrangements are recorded in the Table. Sec/Click and R/Click refer to beam monitor counts or clicks. In our routine calibration method the beam monitor counts are calibrated against a Victoreen ionization chamber in an equilibrium block. Thus, for the 14 MeV electrons scattered by 0.010' Pb + 0.027" Al each beam monitor click represents 6.2 R and the frequency of these clicks gives an estimate of the exposure. It will be seen that our selection of the single scatterer (0.010' Pb + 0.027" Al) is made at the cost of considerable exposure. However the avoidance of technician error in improper scatterer selection compensates for this.

Subsequent donut replacements may necessitate another electron beam flattening method. With the system described here this determination will be simplified greatly over the previous methods available to us.

SUMMARY

A recently improved commercially available film densitometry system enabling convenient selection of depth dose distributions is described. Suitably obtained radiographic films of test exposures in unit density phantoms are densitometered and automatic digital display of the scanned film is produced in about 15 minutes. Examples are shown in which the undesirable bremsstrahlung component of the electron beam is quantified sufficiently for selection of optimum beam flattening arrangements.

ZUSAMMENFASSUNG

Ein kürzlich verbessertes kommerziell erhältliches Film Densitometer System, das eine bequeme Selektion der Tiefen Dosis-Verteilung ermöglicht, wird beschrieben. Geeignet exponierte Filme von Test Bestrahlungen in Phantomen von Standard Dichte werden in dem Densitometer gelesen und eine automatisch digitale Darstellung des untersuchten Films kann in etwa 15 Minuten erhalten werden. Es werden Beispiele gegeben, bei denen die unerwünschte Bremsstrahlungs-Komponente des Elektronenstrahls quantitativ genügend erfasst wird, um eine Selektion optimaler Strahlen Abflachung zu ermöglichen.

RÉSUMÉ

Les auteurs décrivent un système de densitométrie par film récemment perfectionné et commercialisé qui permet une sélection convenable de distribution de dose en profondeur. Des films convenablement radiographiques soumis à des expositions de test dans des fantômes de densité unitaire ont été lus au densitomètre: on obtient en 15 minutes environ une représentation digitale automatique du film exposé. Les auteurs donnent des exemples dans lesquels la composante de bremsstrahlung indésirable du faisceau d'électrons est suffisamment quantifiée pour qu'on puisse choisir les arrangements qui donnent le meilleur aplatissement du faisceau.

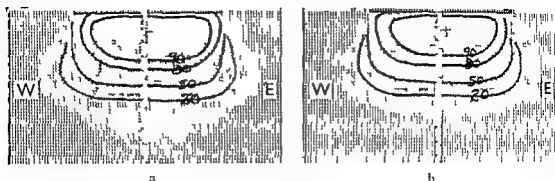


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Ein kurzlich verbessertes kommerziell erhaltliches Film Densitometer System, das eine bequeme Selektion der Tiefen Dosis Verteilung ermöglicht, wird beschrieben. Geeignete exponierte Filme von Test Bestrahlungen in Phantomen von Standard Dichte werden in dem Densitometer gelesen und eine automatisch digitale Darstellung des untersuchten Films kann in etwa 15 Minuten erhalten werden. Es werden Beispiele gegeben, bei denen die unerwünschte Bremsstrahlungs-Komponente des Elektronenstrahls quantitativ genügend erfasst wird, um eine Selektion optimaler Strahlen Abflachung zu ermöglichen.

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REFERENCES

- BOGARDUS C R, WHITE W and POWERS W E An integrated recording film densitometer and isodose plotter *Radiology* 84 (1965), 735
- DUDLEY R A Dosimetry with photographic emulsions *In* *Radiation dosimetry* Vol II 2nd edition Academic Press New York 1966
- SCHULZ R J IHPA/AAPM survey of electron depth dose data *Ann N Y Acad Sci* 161 (1969) 176
- STACY A J, BIVAN A R and DICKENS C W A new phantom material employing depolymerised natural rubber *Brit J Radiol* 34 (1961), 510

KILOVOLTAGE CALIBRATION OF DIAGNOSTIC ROENTGEN RAY GENERATORS

by

JAGDISH P BHATTAGAR and GOPALA U V RAO

With the development of modern shock proof roentgen ray tubes several indirect methods not calling for direct access to the high voltage leads have been proposed for the kilovoltage calibration of roentgen ray machines. Of these the one that uses a crystal monochromator is the most accurate. However, in view of its complexity and unsuitableness for routine measurements a method that is based on the measurement of the intensity of K characteristic radiation resulting from the interaction of a roentgen beam with an interposed radiator is often preferred.

GREENING (1955) first proposed a method based on this principle. In his method the radiator is placed at an angle of 45° to the axis of the incident beam (Fig. 1). An ionization chamber placed at right angles to the roentgen beam measures the 90° secondary radiation exposure rate (R_s) from the radiator. Another chamber is placed in the transmitted beam to measure the transmitted beam exposure rate (R_t). Both the transmitted and secondary radiation exposure rates are measured for various kV settings of the roentgen ray machine. The ratio of R_t/R_s for each kV setting is plotted against the kV setting. GREENING established that in the resulting inverted V shaped curve the peak corresponded to the

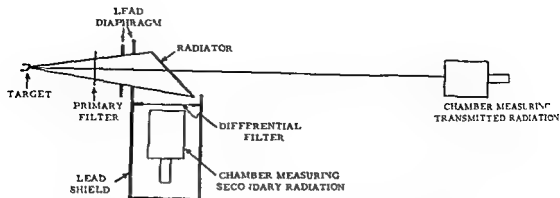


Fig. 1. Experimental arrangement for the kilovoltage calibration of a roentgen unit

actual kV of the K-edge of the radiator. By the choice of several radiators, the calibration can be carried out up to a maximum kilovoltage of 115.6 kV corresponding to the K absorption edge of uranium. A differential filter placed in front of the ionization chamber that measures R_s serves to improve the accuracy of the method.

A few years ago, Bloch & Hale (1966) suggested another method to determine the kV settings corresponding to the K absorption edges of the radiators. In this method, a thin window NaI (Tl) scintillation spectrometer is used instead of an ionization chamber to measure the 90° secondary radiation. The pulse height analyzer of the spectrometer is set with the center of the window at the K characteristic radiation energy of the radiator. The number of counts collected over a given time is plotted as a function of the roentgen tube potential. The resulting plot shows a linear portion which is extended to intercept the tube potential axis (x-axis). The intercept in this method gives the potential of the roentgen tube equivalent to the K absorption edge of the radiator.

Recently, Davison & Reekie (1968) have suggested a method of kV measurement in which only the 90° secondary radiation (R_s) from the radiator is measured by an ionization chamber. The incident primary beam on the radiator is heavily filtered, and the differential filter of Greening is replaced by a thin aluminum filter. R_s is then plotted against the kV meter readings. The kV meter reading at which the K characteristic radiation starts contributing to the 90° secondary radiation is known as the 'breakaway' point. The success of the method therefore depends on the accuracy with which this 'breakaway' point can be determined.

In an attempt to calibrate the kV-meter of a roentgen ray machine, using the methods of Greening (1955), Bloch & Hale (1966), and Davison & Reekie (1968) we found a number of uncertainties associated with each of these meth-

Table
In comparison of kV-calibration methods

Radiator and K absorption edge energy	Differential filter	Thickness of radiator in mm	Kvoltage at the K edge position as detected by the method of		
			GREENING	BLOCH & HALE	SHATYAGAR & RAO
Ta (67.4 keV)	0.15 mm Pb	0.5	71	71.5	69.5
		1.0	74		69.5
Au (89.7 keV)	0.15 mm Pb	0.075	no peak obtained		87.5
		0.125	82		87.5
		0.25	87		87.5
		0.375	87.5		87.5
		0.50	88	84	87.5
Pb (89.0 keV)	0.15 mm Pb	0.125	92		91.0
		0.25	95		91.0
		0.375	96		91.0
		0.50	96.5		91.0
		0.625	97		91.0
		0.75	97.5		91.0
		1.00	98	93	91.0
U (113.6 keV)	0.26 mm U	0.264	121		121.5
		0.578	124		121.5

ods In this paper we propose to discuss these uncertainties and suggest a method which gives accurate results to within $\pm 1\%$ up to 115 kV. Theoretical justification in support of the proposed method will also be presented.

The roentgen rays used to obtain the data reported in this paper were produced by a Siemens constant potential generator operated in the range of 60–120 kV. The experimental set up used for the study is shown in Fig. 1. The radiation detectors used in most of our experiments for measuring the transmitted and the 90° secondary radiation exposures were standard Victoreen R chambers coupled to a Townsend balance circuit. The radiators used were Ta, Au, Pb, and U. Their thicknesses and K absorption edge energies are given in the Table in which the differential filters used and their thicknesses are also given.

In order to repeat the BLOCH & HALE (1966) method a thin window (27 mg)

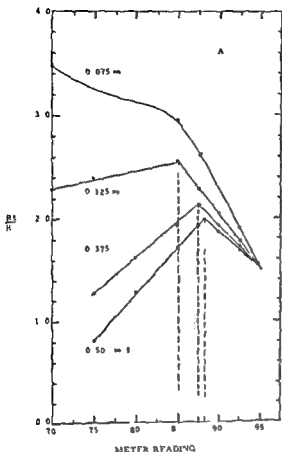


Fig 2 Results obtained with the GREENING (1955) method for various thicknesses of a gold radiator. There is no peak in evidence at a thickness of 0.075 mm and for higher thicknesses the peak goes on shifting towards higher kV meter readings.

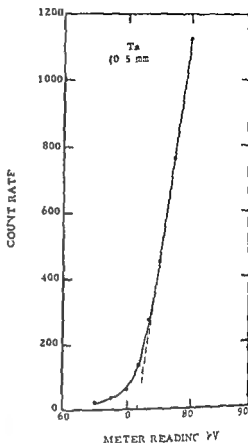


Fig 3 Results obtained with the BLOCH & HALE (1966) method for Ta radiator (0.5 mm thick). Intercept of dotted line with kV axis is expected to yield the K absorption edge of the radiator but in practice this intercept occurs at a value considerably higher than the K absorption edge.

cm Al) NaI (TI) scintillation spectrometer with a single channel analyzer was used. The scintillation spectrometer was calibrated using standard gamma sources of ^{109}Cd (22 keV), ^{133}Ba (32 keV) and ^{160}Tm (84 keV). A window width of 2 keV was used.

Results

The Greening method The results obtained using the GREENING (1955) method for various thicknesses of a gold radiator are given in Fig 2. For convenience, the values of R_2/R_1 are normalized at a meter reading of 95 kV. This figure clearly illustrates how the position of the inverted V peak depends considerably

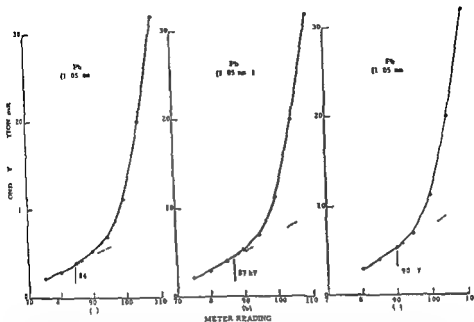


Fig 4 Results obtained with the DAVISON & REAIZE (1969) method for a lead radiator. Three different straight lines drawn through the same set of data yield three different breakaway points at 84, 87 and 90 kV. The 75 kV data point in (c) is deleted purposely to demonstrate that the position of the 'breakaway' point depends on the kV setting at which the measurements are started.

on the thickness of the radiator used. The peak is obtained at different values of kV for different radiator thicknesses. Furthermore, at a thickness of 0.075 mm or less there does not exist a peak at all. The data obtained with all the radiators used in our study are summarized in the Table p 557. It is seen that in all cases the position of the peak keeps on shifting further and further away towards the high voltage side with increase in thickness of radiator.

This shift of peak position seems to occur because of the variation of R_t with increase in radiator thickness. Closer investigation showed that while R_s initially increases and soon reaches a saturation value at some critical thickness, R_t on the other hand decreases steadily with radiator thickness. (In the case of gold and lead the critical thickness was found to be 0.2 mm and in the case of tantalum and uranium the minimum thicknesses available to us were not small enough to detect this variation.) Moreover, the shape of R_t kV curve was observed to change significantly with radiator thickness. Therefore, when the ratio R_t/R_s is plotted against kV for different radiator thicknesses, one observes a shift in peak position mainly due to the variation in R_t .

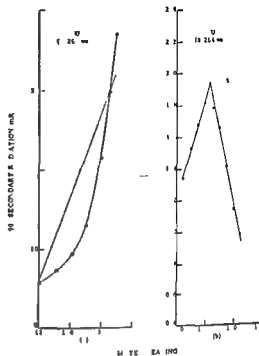


Fig 5 Diagram illustrating the procedure proposed for an accurate determination of the K absorption edge. An arbitrary straight line is drawn on the R_s kV curve in such a manner as to intersect it at points as far separated as possible. For different kV settings the ratios of the ordinates of points on the straight line to those on the R_s kV curve are plotted as a function of kV setting (b). The K absorption edge is indicated by the peak of the inverted V shaped curve that results.

The Bloch & Hale method A typical result obtained with the Bloch & Hale (1966) method for Ta radiator (0.5 mm thick) is recorded in Fig 3. A comparison of results obtained by this method for Ta (0.5 mm thick), Au (0.5 mm thick) and Pb (1.05 mm thick) with the Greening method at these thicknesses is given in the Table. It is seen that the values obtained by the Bloch & Hale method are different from those obtained by the Greening method at the thicknesses under consideration. Additionally, the former method is open to question (DAVISON & REEKIE 1968) due to the fact that the response of a scintillation detector spreads the monochromatic radiation into a Gaussian type of distribution, and consequently the counts taken from a window set to the characteristic radiation energy of the radiator will not only be due to characteristic radiation but also due to non characteristic scatter from the radiator. For these reasons the intercept of the extrapolated portion of the kV count rate curve with the kV axis (Fig 3) is not likely to indicate the correct K absorption edge of the radiator. In general, the intercept occurs at a value higher than the K absorption edge of the radiator.

The Davison & Reekie method Using the DAVISON & REEKIE (1968) method we experienced considerable difficulty in deciding the points through which a straight line should be drawn to find out the 'breakaway' point. It may be seen from Fig 4 that three different straight lines can be drawn through the same

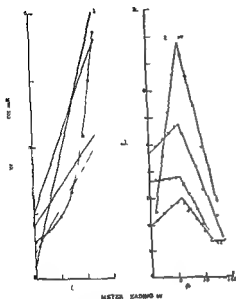


Fig. 6. Diagram illustrating that the position of the peak of the inverted V shaped curve is independent of the slope of the arbitrary straight line.

et of data giving the breakaway points at 84, 87, and 90 kV. It is not known which of these three breakaway points is the correct one. In Fig. 4c the 75 kV data point is deleted purposely to demonstrate that the position of the breakaway point depends on the kV setting at which the measurements are started. Similar difficulty was experienced in the case of all other radiators. It thus seems that the Davison & Reekie method of finding out the breakaway point for a radiator could largely depend on an individual's personal judgement. This difficulty arises from the fact that the 90° secondary radiation is not a linear function of the applied kilovoltage. It has a definite curvature and there can be drawn any number of straight lines by observation through points lying on a curve.

Proposed modification of Greening's method

The difficulties encountered with the methods discussed above can be overcome by the following procedure:

On a plot of the 90° secondary radiation R_s (as measured by an ionization chamber) against the kV setting of the machine (Fig. 5a) an arbitrary straight line SL is drawn. If now for different kV settings the ratios of the ordinates of points on the straight line to those on the R_s kV curve are plotted as a function of the kV setting, an inverted V shaped peak results (Fig. 5b). The position of the peak occurs at the point where the trend of the R_s kV curve changes due to

the addition of the K characteristic radiation from the radiator. The position of this peak is independent of the slope of the straight line as illustrated in Fig. 6. This in fact, is what one would expect, as can be seen from the following.

The 90° secondary radiation exposure rate, $R_s(V)$, at any kilovoltage V is given by the equation

$$R_s(V) = I_{\text{coh}}(V) + I_{\text{incoh}}(V) + I_{\text{ch}}(V) \quad (1)$$

where $I_{\text{coh}}(V)$ and $I_{\text{incoh}}(V)$ are the exposure rate components due to the coherent and incoherent scattering respectively and $I_{\text{ch}}(V)$ is the component due to the characteristic radiation from the radiator.

At tube voltages less than the K-absorption edge of the radiator, eq. (1) simply reduces to

$$R_s(V) = I_{\text{coh}}(V) + I_{\text{incoh}}(V) \quad (2)$$

Now if $Y = mV + C$ is assumed to be the equation for the arbitrary straight line drawn on the R_s kV curve, it follows that the ratio Q of the ordinate of this straight line to that of the R_s kV curve at any tube voltage V is given by

$$Q = \frac{mV + C}{I_{\text{coh}}(V) + I_{\text{incoh}}(V)} \quad \text{when } V < V_K \quad (3)$$

$$Q = \frac{mV + C}{I_{\text{coh}}(V) + I_{\text{incoh}}(V) + I_{\text{ch}}(V)} \quad \text{when } V > V_K \quad (4)$$

where V_K is the voltage corresponding to the K absorption edge of the radiator.

Assuming that the arbitrary straight line is drawn above the R_s kV curve as in Fig. 5a and in Fig. 6a, it follows from eq. (3) that Q is an increasing function of V as long as V is less than V_K . This is because, in this region, the numerator, which is the ordinate of the arbitrary straight line increases much more rapidly than the sum of the terms $I_{\text{coh}}(V)$ and $I_{\text{incoh}}(V)$ in the denominator. However as soon as V becomes greater than V_K , Q begins to decrease with V because of the sudden addition of the rapidly increasing term $I_{\text{ch}}(V)$ to the denominator (eq. 4 and Fig. 7). Hence the peak of the inverted V shaped curve of Q versus kV always occurs at the K absorption edge of the radiator regardless of the slope of the arbitrary straight line. In practice, for greater accuracy we found that the following criteria should be observed while drawing the arbitrary straight line. First of all, care should be taken to draw the straight line sufficiently above the R_s kV curve. Secondly, it should be drawn in such a way as to cut the R_s kV curve at two points, one point being on the low kV side where the increase of R_s is slow and the other point on the high kV side where R_s increases rapidly with kV.

The Table, p. 557, shows the results obtained by this method of kV calibra-

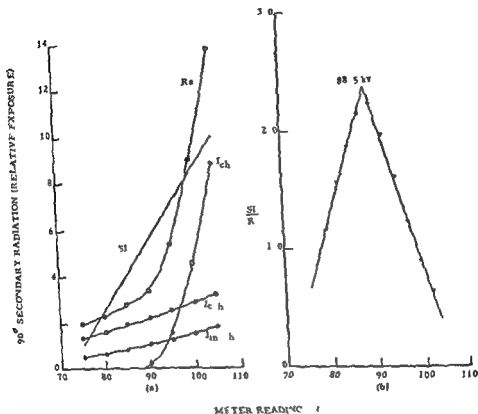


Fig. 7 Theoretical confirmation of the straight line method of kV calibration. In (a) are shown the values of I_{in} , I_{ch} and I_{c+h} as a function of tube voltage calculated theoretically for a 2 mm Al primary filter and a 0.15 mm lead differential filter. $R_s \approx I_{in} + I_{ch} + I_{c+h}$. In (b) the straight line method is applied to these theoretical values of R_s . The peak of the resulting V-shaped curve is seen to occur at 88.5 kV while the K absorption edge energy of lead is 88 keV.

tion for a number of radiator thicknesses. It will be seen from this table that for the entire range of thicknesses used with gold the position of the peak occurred at 82.5 kV. Likewise the peak always occurred at 91.0 kV for lead. Similarly in the case of tantalum and uranium quite satisfactory results were obtained.

The accuracy with which the K absorption edge of a radiator can be determined by the method outlined above can be tested in one of two ways. The first one is to make a direct check with a sphere gap. An alternative method is to make a theoretical estimate of R_s for different values of kV in the case of a typical radiator and then use it to estimate its K absorption edge by the method under test. The second method was chosen because of the inaccessibility to the high

the addition of the K -characteristic radiation from the radiator. The position of this peak is independent of the slope of the straight line as illustrated in Fig. 6. This in fact, is what one would expect, as can be seen from the following.

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$$Q = \frac{mV + C}{I_{\text{coh}}(V) + I_{\text{incoh}}(V)} \quad \text{when } V < V_{\text{ic}} \quad (3)$$

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where V_{ic} is the voltage corresponding to the K absorption edge of the radiator.

Assuming that the arbitrary straight line is drawn above the R_s kV curve as in Fig. 5a and in Fig. 6a, it follows from eq. (3) that Q is an increasing function of V as long as V is less than V_{ic} . This is because, in this region, the numerator, which is the ordinate of the arbitrary straight line increases much more rapidly than the sum of the terms $I_{\text{coh}}(V)$ and $I_{\text{incoh}}(V)$ in the denominator. However, as soon as V becomes greater than V_{ic} , Q begins to decrease with V because of the sudden addition of the rapidly increasing term $I_{\text{ch}}(V)$ to the denominator (eq. 4 and Fig. 7). Hence the peak of the inverted V shaped curve of Q versus kV always occurs at the K absorption edge of the radiator regardless of the slope of the arbitrary straight line. In practice, for greater accuracy, we found that the following criteria should be observed while drawing the arbitrary straight line. First of all, care should be taken to draw the straight line sufficiently above the R_s kV curve. Secondly it should be drawn in such a way as to cut the R_s kV curve at two points, one point being on the low kV side where the increase of R_s is slow and the other point on the high kV side where R_s increases rapidly with kV.

The Table, p. 557, shows the results obtained by this method of kV calibration.

$$I_{\text{ch}} (V) = 8.789 \int_0^V (1-E) [F/Z]^2 E (\mu_{\text{en}}/\rho)_{\text{air}} \exp(-\mu_p l - \mu_k x) dE$$

$$I_{\text{ch}} (V) = 1.15 \times 10^{12} \int_0^V \frac{(1-E)}{E} \tau E_k (\mu_{\text{en}}/\rho)_{\text{air}} \exp(-\mu_p l - \mu_k x) dE$$

- where V = maximum photon energy present in the primary beam (equal to the applied kV) in keV
 E = photon energy less than V in keV
 E = energy of the photons scattered at 90° in keV
 $d\sigma/d\Omega$ = Klein-Nishina differential cross section in $\text{cm}^2/\text{electron}$ per unit solid angle at 90°
 S = reduction factor to correct $(d\sigma/d\Omega)$ for bound electrons (Also known as incoherent scattering function)
 μ , μ , μ_k = linear attenuation coefficients in cm^{-1} of differential filter for photon energy E scattered photon energy E and K -characteristic radiation energy E_k respectively
 x = thickness in cm of the differential filter
 μ_p = linear attenuation coefficient in cm^{-1} of the filter in the primary roentgen beam for photon energy E
 l = Thickness in cm of the filter in primary roentgen beam
 $\tau (\mu_{\text{en}}/\rho)_{\text{air}}$ = mass energy absorption coefficient in cm^2/g for air for photon energy E
 $\tau (\mu/\rho)_{\text{air}}$ = mass energy absorption coefficient in cm^2/g for air for scattered photon energy E and K -characteristic photon energy E_k
 $\tau (\mu/\rho)_{\text{air}}$ = mass energy absorption coefficient in cm^2/g for air for scattered photon energy E and K -characteristic photon energy E_k
 $[F/Z]$ = atomic structure factor per electron of the radiator of atomic number Z
 τ = photoelectric cross-section for the entire atom in cm^2/atom
 E_k = K absorption edge energy of the radiator in keV (88 keV for the lead radiator)

Using the appropriate values of $d\sigma/d\Omega$, S , μ , τ (CRODSTEIN 1957), $(\mu_{\text{en}}/\rho)_{\text{air}}$ (ICRU 1962), F/Z (VELMS & OPPENHEIM 1955), τ and E_k (FRIE & HANDEE 1955) we estimated the relative values of I_1 , I_2 , I_3 and I_4 by numerical integration. The results are shown in Fig. 7a in the case of a lead radiator.

Acknowledgements

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SUMMARY

The uncertainties associated with the current methods of kV-calibration of diagnostic roentgen ray generators making use of the K absorption edges of different elements are discussed. A method of eliminating these uncertainties is proposed. The proposed method is based on a measurement of the 90° secondary radiation (R_1) from an interposed radiator in the primary roentgen beam at different kV settings of the roentgen ray generator.

used in these theoretical calculations is given in the appendix. The values of R_s calculated in this manner for a lead radiator were plotted as a function of kV (see Fig. 7) and the procedure outlined above applied to this theoretical R_s kV curve. When this was done, an inverted V-shaped curve resulted, with a peak at 88.5 kV. Since the K absorption edge of lead was assumed to be 88 keV in the theoretical calculations, it follows that the value obtained by the straight line method is within 1% of the true value. It may therefore be expected that a determination of the K absorption edge by this method from an experimentally determined R_s kV curve is also equally accurate.

Conclusion

Experimental and theoretical evidence in support of an accurate method for the kilovoltage calibration of diagnostic roentgen ray units, based on a measurement of the secondary radiation alone from an interposed radiator, is presented. The method is essentially a modification of Greening's original method. While in Greening's method, the ratio R_t/R_s is plotted against kV, in our case the measurement of R_t is altogether eliminated and the numerator is replaced by a linear function of kV. The transmitted radiation exposure rate that Greening uses in the numerator is known to be a power function of the voltage and varies with the thickness of the radiator. The calibration is therefore subject to unpredictable errors depending on the thickness of the radiator. In our method, since the R_t kV curve is replaced by a simple straight line, these uncertainties no longer exist. The modified procedure suggested here is especially suited for day to day field work and is worthy of consideration for being developed into a single package kilovoltage calibration unit.

Appendix

Theoretical calculation of secondary radiation from a radiator

Consider a lead radiator of thickness dt placed in a roentgen beam. Let the secondary radiation from it be filtered by a differential lead filter of thickness l cm. The resultant 90° secondary radiation exposure R_s (V) at any kilovoltage v is given by the equation

$$R_s(V) = I_{\text{coh}}(V) + I_{\text{incoh}}(V) + I_{\text{ch}}(V)$$

where $I_{\text{coh}}(V)$ and $I_{\text{incoh}}(V)$ are the components due to coherent and incoherent scattering respectively and $I_{\text{ch}}(V)$ is the component due to characteristic radiation.

Making use of the well known equation of KRAMER (1923) for the spectrum of the primary roentgen beam incident on the radiator it can be shown that I_{coh} , I_{incoh} and I_{ch} for a lead radiator are given by the following expressions

$$I_{\text{incoh}}(V) = 0.275 \times 10^{25} \int_0^1 \frac{1-E}{E} \left(\frac{d\sigma}{d\Omega} \right) S E^{-F} (\mu_{\text{en}}/\rho)_{\text{air}} \times \frac{510+E}{510-E} \exp(-\mu_p l - \mu_K \kappa) dE$$

TECHNETIUM 99m SULFIDE COLLOID PREPARATION FOR SCINTIGRAPHY OF THE RETICULOENDOTHELIAL SYSTEM

by

R. BERTIL R. PERSSON and YNGVE NAVERSTEN

The technetium ^{99m}Tc was first used by HARPER (1964) in a fat emulsion and later as a sulfide colloid for liver scintigraphy. In 1966 HARPER et coll prepared the sulfide colloid by introducing H_2S gas into an acidified pertechnetate solution in the presence of gelatin. This method was somewhat complicated for routine use and the yield was a mixture of colloidal and soluble technetium. Simplified methods for the preparation of ^{99m}Tc sulfide colloids have been developed by PATTON et coll (1966), STERN et coll (1966) and LARSSON & NELP (1966). The method described by PATTON et coll employed perthenate ($\approx 0.002 \text{ M}$) as the carrier of pertechnetate and formed the sulfide colloid in the presence of thiosulfate ($\approx 0.010 \text{ M}$) at a low pH value (≈ 1) and heated to about 100°C . The experimental conditions for this method have been investigated by LARSSON & NELP and are well known. STERN et coll found that if perthenate was excluded and the pH value readjusted beyond 6.5 the dissociation of the colloid would take place.

Different agents have been used to stabilize the colloid e.g. gelatin by HARPER

ZUSAMMENFASSUNG

Die mit den gängigen Methoden der kV Kalibrierung von diagnostischen Röntgenapparaten verbundenen Unsicherheiten bei der Anwendung der K_α Absorptionskanten verschiedener Elemente werden diskutiert. Eine Methode diese Unsicherheiten zu eliminieren wird vorgeschlagen. Die vorgeschlagene Methode gründet sich auf eine Messung der 90° Sekundär Strahlung (R_s) von einem in den primären Röntgen Strahl bei verschiedener kV Einstellung des Röntgenapparates gebrachten Strahlers.

RÉSUMÉ

Les auteurs étudient les incertitudes que comportent les méthodes habituelles d'étalonnage de la tension des générateurs de radio diagnostic par l'utilisation des pics de l'absorption K_α de différents éléments. Ils proposent une méthode qui élimine ces incertitudes elle est basée sur la mesure du rayonnement secondaire (R_s) émis à 90° par une source secondaire interposée dans le faisceau du rayon primaire pour différentes tensions du générateur de rayon de roentgen.

REFERENCES

- BLOCH P and HALL J. Calibration of potential of x ray generators in the diagnostic energy range. *Phys in Med Biol* 11 (1966) 577
- DAVISON M and REELIF D. Measurement of peak voltage of diagnostic x ray generators. *Phys in Med Biol* 13 (1968) 643
- FINE S and HANDEE C T. X ray critical absorption and emission energies in keV. *Nucleonics* 13 (1955) 36
- GREFNING J R. The measurement by ionization methods of the peak kilovoltage across x ray tubes. *Brit J appl Phys* 6 (1955) 73
- GRODSTEIN C W. X ray attenuation coefficients from 10 keV to 100 meV. *NBS Circular* No 583 (1957)
- KRAMER H A. On the theory of the x ray absorption and of the continuous x ray spectrum. *Phil Mag* 46 (1923) 836
- NEIMS A T and OPPENHEIM I. Data on the atomic structure factors: computation and survey. *NBS J Res* 55 (1955) 53

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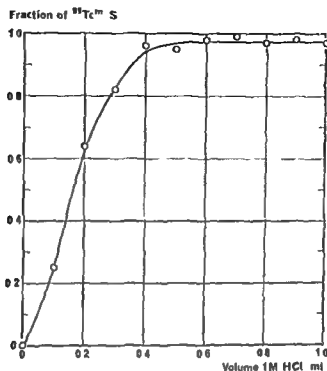


Fig 1 The fraction of $^{99}\text{Tc}^m$ retained as sulfide colloid after filtration through a $0.45 \mu\text{m}$ millipore (HA) filter when varying volumes of 1 M HCl were added to the stock solution before heating for 3 min in a boiling water bath

et coll and by PATTON et coll, dextran by IERSSON & NÄVERSTEN and by DWORIN et coll (1967), carboxymethyl cellulose (CMC) by STERN et coll and polyvinylpyrrolidone (PVP) by GARZON et coll (1966), SZYMENDERA et coll (1969) and EGF & RICHARDS (1969). A survey of 29 institutions in the U.S.A. conducted by SMITH et coll (1967) revealed anaphylactic and anaphylactoid reactions in 0.9% of patients given $^{99}\text{Tc}^m$ sulfide colloid stabilized with dextran. These results were confirmed by the finding of BAILEY et coll (1967) that high molecular dextran induces anaphylaxis acting as a non-specific histamine releaser in patients pre-sensitized to native dextran. With gelatin as a stabilizing agent, however, fewer than 0.1% reactions were reported by SMITH et coll and these could not be definitely associated with the colloid. WEBBER et coll (1969) eliminated the risk related to the use of dextran by preparing the colloid without a stabilizer. They obtained however a suspension, not a colloid, and as this precipitates it was necessary to resuspend it by shaking before administration. They are now looking for a nonallergenic stabilizer because the amount of soluble technetium increases when left standing several hours after its preparation. The effect on the colloid by omitting a stabilizer has also been studied by LOPEZ &

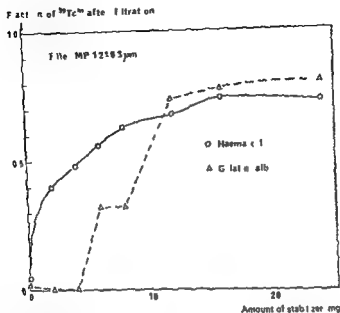


Fig. 7 The ratios between the ^{99m}Tc radioactivity concentration (mCi/ml) after and prior to filtration through a millipore filter 12 μm with various amounts of Haemacel or Gelatin Alba as stabilizing agent

FRANCK (1960) they too observed some aggregation of the colloid particles in vitro but no significant uptake in the lungs was evident in the scintigrams. By reducing the amount of sodium thiosulfate to one tenth they got no measurable precipitation but the amount of free ^{99m}Tc increased from 2% directly after the preparation up to 4.2% five hours later.

We wanted to further improve the method described by PATTON et coll. and by LARSSON & NEIF and we decided to use gelatin as a stabilizing agent. Two pharmaceutical materials were available: Gelatin Alba (USA) and Haemacel. The latter is a commercially available plasma expander free from pyrogenic and antigenic reactions as reported by SCHERER & WUNSCH (1963) and by HASSIG (1966).

The following chemicals and reagents were used in the preparation of the ^{99m}Tc sulfide colloid:

Chemicals

Sodium thiosulfate pro analysis (Merck, Germany) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ Mol. wt. 248.18

Technetium 99m generator (The Radiochemical Centre, Amersham, England) code MCC 2

Sodium phosphate pro analysis (Merck, Germany) $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ Mol. wt. 260.00

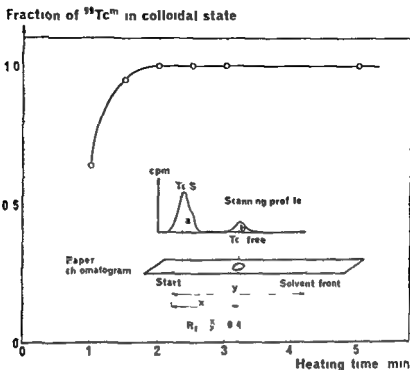


Fig. 3 Fraction of ^{99m}Tc activity incorporated in colloidal sulfide particles after heating in boiling water at various time intervals

Potassium perrhenate (Hopkin & Williams Ltd England) KReO_4 Mol wt 289.32
 Hydrochloric acid pro analysi (Riedel-DeHaen AG Germany) HCl Mol wt 36.47 37%
 Haemacel (Farbwerke Hoechst AG Germany) gelatin polymerizate 3.5% solution
 ave Mol wt 35 000

Gelatin Alba (USP) gelatin powder for pharmaceutical use

Sodium chloride (ACO Sweden) NaCl 0.9% for infusion

Reagents

Stock solution 0.600 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ 0.125 g of KReO_4 18 ml Haemacel 3.5%
 sterile water added to 100 ml

A sterile kit for 10 preparations of Tc S consists of 10 stock solutions each 2 ml in 20 ml
 bottles 1 bottle 1 M hydrochloric acid 10 ml 1 bottle 0.2 M Na_2HPO_4 solution 50 ml

Procedure The ^{99m}Tc eluate from the generator was obtained in 5 ml fractions of 0.9% NaCl in multidose bottles and sterilized at 120°C for 20 minutes. One fraction was transferred to a 20 ml multidose bottle containing 2 ml of the stock solution. The colloid was formed by heating in a boiling water bath for 30 minutes. After cooling, the acidity was adjusted to $\text{pH} \approx 6.4$ by adding 4.0 ml of 0.2 M Na_2HPO_4 . The Tc S colloid was then ready for clinical use.

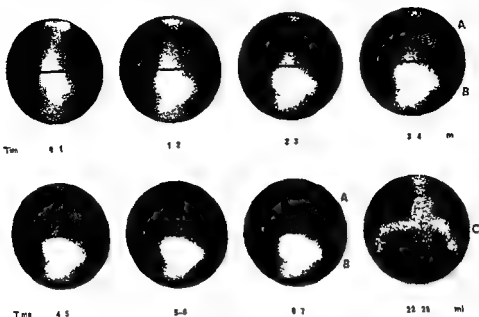


Fig 4 Gamma camera photographs obtained at different times after intravenous administration of Tc S colloid in the ear vein of a rabbit in supine position. The upper (cranial) half of the view is called field A in the text and the lower (caudal) half field B. The picture in the lower right corner is the pelvis and backbone in the same position. Exposure time 4 minutes. termed field C in the text.

The normal activities to be used for liver and bone marrow scintigraphy are 2—3 mCi and 5—10 mCi respectively.

This procedure differs from that described by PATTON *et coll* and by LARSEN & NELP by the use of only 0.5 ml 1 M HCl instead of 10 ml. It was thus possible to neutralize the solution with only 4 ml 0.2 M Na HPO₄ in place of 3 ml of the concentrated buffer mixture suggested by these authors and this resulted in an easily prepared solution and an almost isotonic final product. Furthermore the use of Haemacel as stabilizing agent excludes the risk of pyrogenic and antigenic reactions associated with the stabilizer.

In vitro tests of the colloid

Filtration tests The efficiency of the colloid formation was investigated with different amounts of 1 M HCl added to the stock solution before heating in boiling water for 3 minutes. After pH adjustment 500 μ l were taken as a reference and the rest was passed through a U 45 μ m millipore (HA) filter. The

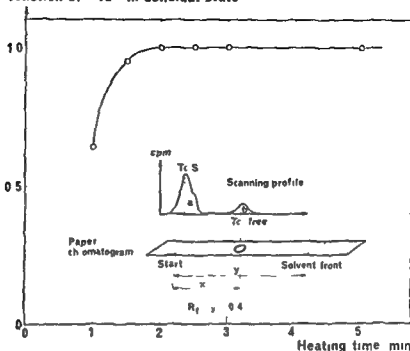
Fraction of $^{99}\text{Tc}^m$ in colloidal state

Fig. 3 Fraction of $^{99}\text{Tc}^m$ activity incorporated in colloidal sulfide particles after heating in boiling water at various time intervals

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Reagents

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A sterile kit for 10 preparations of Tc S consists of 10 stock solutions each 2 ml in 20 ml bottles, 1 bottle 1 M hydrochloric acid 10 ml 1 bottle 0.2 M Na_2HPO_4 solution 50 ml

Procedure The $^{99}\text{Tc}^m$ eluate from the generator was obtained in 5 ml fractions of 0.9% NaCl in multidose bottles and sterilized at 120°C for 20 minutes. One fraction was transferred to a 20 ml multidose bottle containing 2 ml of the stock solution. The colloid was formed by heating in a boiling water bath for 30 minutes. After cooling, the acidity was adjusted to $\text{pH} = 6.4$ by adding 4.0 ml of 0.2 M Na_2HPO_4 . The Tc S colloid was then ready for clinical use.

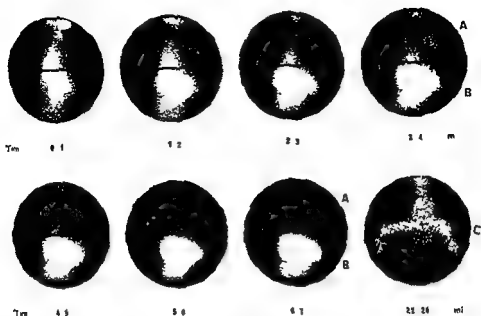


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Table 1

Ratio between number of counts in field A (cranial) and field B (caudal) with different amounts of Gelatin Alba or Haemacel

Amount mg	Gelatin Alba counts in A/counts in B	Haemacel counts in A/counts in B
0	0.056	0.056
12	0.165	0.074
16	0.092	0.074
24	0.074	0.076

Table 2

Ratios between number of counts in lung field A and liver field B and ratios between number of counts in pelvic field C and liver field B at varying heating times during the preparation of the Tc S colloid

Heating time (100 °C) min	Ratio counts in A/counts in B	Ratio counts in C/counts in B
2	0.072	0.071
3	0.089	0.083
5	0.055	0.087
7	0.100	0.103
10	0.069	0.075
Mean \pm S D	0.077 \pm 0.020	0.084 \pm 0.014

fraction of ^{99}Tc S stopped by the filter, as a function of the volume 1 M HCl added, is given in Fig. 1.

When more than 0.4 ml 1 M HCl was added, nearly all the colloidal particles were stopped by the filter, but if less 1 M HCl was added, the $^{99}\text{Tc}^{\text{m}}$ was not fixed quantitatively in the colloidal state. To obtain a moderate volume of the preparation, we decided to use 0.5 ml 1 M HCl.

The colloid size was also studied with varying amounts of stabilizing agent. It was prepared as described above except that the amounts of Haemacel and Gelatin Alba in the stock solution were varied. The colloid one hour after the preparation was passed through a 1.2 μm millipore (RA) filter. Aliquots were taken and the results are given in Fig. 2 as the ratio of $^{99}\text{Tc}^{\text{m}}$ activity concentration after and prior to filtration. This investigation indicated that a small amount of Haemacel is sufficient to produce a stabilization effect on the colloid. As more than 12 mg is without further effect, this amount was employed. Small quantities of Gelatin Alba failed to have as good a stabilizing effect as Haemacel.

Table 3

Ratio between number of counts in field A (cranial) and field B (caudal) with varying time intervals between the preparation and administration of the Tc-S colloid

Time interval between preparation and administration	Counts in A/counts in B
15 minutes	0.047
4 hours 30 minutes	0.041
7 hours 15 minutes	0.035

put in excess of 12 mg produced about the same result. The efficiency of colloid formation as a function of heating time was investigated with paper chromatography. A small volume (100 μ l) was placed on Whatman paper No. 1, the lower part of which was immersed in 85% methanol solution for 6 hours. The paper then was scanned with a slit collimated $\phi 3 \times 2$ NaI(Tl) crystal (Fig. 3). The results obtained after varying the heating time are given in the figure as the fraction $a/(a+b)$ of ^{99m}Tc activity incorporated in the colloidal particles. A plateau was obtained after 2 minutes heating but 30 minutes were adopted for routine use.

Colloidal particle size. One drop of the colloid was spread on a glass plate and after drying was viewed in a light microscope through immersion oil. The mean size of the colloidal particles was estimated to be $0.6 \pm 0.2 \mu\text{m}$. Another drop was put on a copper grid and dried for 30 minutes before it was viewed in a electron microscope. The mean size was estimated from photographs ($16,000\times$ magnification) as $0.8 \pm 0.2 \mu\text{m}$.

In vivo tests of the colloid

The uptake of the Tc-S colloid by the liver and bone marrow was tested in rabbits supine under a gamma camera (Nuclear Chicago Pho/Gamma III). Sequential scintigrams were then obtained after intravenous injection of the colloid into the ear vein.

A Tc-S colloid of good quality is mainly taken up by the liver and the rest by the spleen and bone marrow. If however the colloid consists of particles or aggregates larger than $7 \mu\text{m}$ they will be trapped in the capillary bed of the lung. The field viewed by the gamma camera was therefore divided into two parts: one part A, viewing the cranial part with the chest, and the other part B, the caudal part including the liver. A scintigram was obtained every minute

after administration and the counts were registered separately for fields A and B. The ratio (counts in A/counts in B) registered 15 minutes after administration was used as an index of the quality of the colloid. This method is similar to that described by DWORKIN *et coll.* who used the liver to blood pool (L/BP) ratio as an index of the quality of the colloid in man. A series of photographs with the border between the fields marked with a black line, are given in Fig. 4. As the colloid also accumulates in bone marrow, the pelvis and backbones could be seen when the caudal part of the body, except the liver, was viewed by the undivided gamma camera field C. As may be seen from Fig. 4, the bone marrow accumulation was approximately the same in fields A and C. The ratios (counts in C/counts in B, and counts in A/counts in B) would therefore be roughly the same if no particles were trapped by the lungs.

The gamma camera has been used for studying the influence of the stabilization agent on the quality of the Tc-S colloid. The ratios (counts in A/counts in B) established after 15 minutes are given in Table 1.

No significant variation of the quality was found with or without stabilizer. This confirms the finding by WEBBER *et coll.* (1969) and LOPEZ & FRENCH (1969) that there was no significant uptake in the lungs. We also studied the use of a reduced amount of $\text{Na}_2\text{SO}_3 \cdot 5\text{H}_2\text{O}$ (1.6 mg) and omitted both the KReO_4 and the stabilizer, as suggested by LOPEZ & FRENCH. This preparation was administered to a rabbit viewed by the gamma camera. The quality value obtained was 0.204, which is significantly higher than for the standard preparation. No uptake was observed in the lungs but the increased level of free TcO_4 could possibly explain this figure.

The quality of the Tc-S colloid also depends on the time interval in boiling water during which the colloid is formed. Colloids were therefore prepared at different time intervals, and investigations were carried out in rabbits with a gamma camera as above. The counts in A/counts in B, and counts in C/counts in B ratios are given in Table 2. There were no great differences in the quality of these preparations with heating intervals varying from 2 to 10 minutes.

The stability of the Tc-S colloid was also tested by administering it to rabbits at different times after the preparation and by studying the liver uptake. The ratios (counts in A/counts in B) are given in Table 3. The conclusion of this test is that no significant change of the colloid occurred during the day. This fact was also confirmed by filtration tests.

Conclusion

The aim of this investigation was to make a Tc-S colloid for liver and bone marrow scintigraphy free from harmful reactions. The preparation procedure

was studied in detail. The use of Haemaccel as stabilization agent gives reproducible preparations without any potential risk of pyrogenic or anaphylactic reactions. In vivo tests in rabbits indicate that the Tc S colloid is a good agent for liver and bone marrow scintigraphy and has now replaced the ^{199}Au colloid in our department. The chemicals are delivered in sterile kits from the pharmacy and the Tc S colloid is prepared by a hospital physicist. Clinical experience so far is limited but the method has worked out satisfactorily in more than 800 patients including several repeated examinations at different time intervals.

Acknowledgements

The authors wish to express their deep gratitude to their collaborators too numerous to name individually for their great help in the various stages of the work.

SUMMARY

A method of preparing a $^{99}\text{Tc}^m$ sulfide colloid for bone marrow and liver scintigraphy is described, the aim being to achieve a preparation free from harmful reactions. The uptake of the colloid in the bone marrow and liver was investigated in rabbits with a gamma camera. The preparation is now in clinical use.

ZUSAMMENFASSUNG

Eine Methode zur Herstellung eines Kolloidpräparates von $^{99}\text{Tc}^m$ Sulfid für Knochenmark und Leberszintigraphie wird beschrieben. Das Ziel der Arbeit war ein Präparat zu produzieren, das frei von schädlichen Nebenwirkungen ist. Die Aufspeicherungsrate des Kolloids in Leber und Knochenmark von Kaninchen wurde mit Hilfe einer Gamma-Kamera bestimmt. Das Präparat wird jetzt klinisch benutzt.

RÉSUMÉ

Les auteurs décrivent une méthode de préparation de sulfite de $^{99}\text{Tc}^m$ pour la scintigraphie de la moelle osseuse et du foie, le but étant de faire une préparation qui ne donne pas de réaction nuisible. Ils ont étudié la fixation de ce colloïde dans la moelle osseuse et dans le foie sur des lapins au moyen d'une gamma camera.

REFERENCES

1. BAILEY G., STRUB R. L., KLEIN R. C. and SALVAGGIO J. Dextran induced anaphylaxis. *J Amer med Ass* 200 (1967) 889.
2. DWORKIN H. J., NELLS Z. and DOWSE L. Rectilinear liver scanning with technetium 99m sulfide colloid. *Amer J Roentgenol* 101 (1967) 557.
3. EGE G. N. and RICHARDS L. P. Introducing PVP as a stabilizer in the preparation of technetium sulfur colloid. *Brit J Radiol* 42 (1969) 552.

- 4 GARZON O. L., PALCOS M. G. and RADICELLA R. A technetium 99m labelled colloid
Int J appl Radiat 16 (1965), 613
- 5 HARPER P. V. Improved liver scanning with 6 hour Tc 99m in fat emulsion J nucl
Med 5 (1964), 382
- 6 — LATIROP K. A. JIMENEZ F. et coll. Technetium 99m sulfur colloid In Radioactive
pharmaceuticals p 343 Edited by G. A. ANDREWS R. M. KNISELEY and H. N.
WAGNER U. S. Atomic Energy Commission CONF 651111 (1966)
- 7 HASSIG A. Plasmaexpander Helv chir Acta 33 (1966) 335
- 8 LARSSON S. M. and NELP W. B. Radiopharmacology of a simplified technetium 99m
colloid preparation for photoscanning J nucl Med 7 (1966) 817
- 9 LOPEZ R. B. and FRENCH R. J. Stabilizer free technetium colloid Brit J Radiol 42
(1969), 663
- 10 PATTON D. D. GARCIA E. N. and WEBBER M. M. Simplified preparation of technetium
sulfide colloid for liver scanning Amer J Roentgenol 97 (1966) 880
- 11 SCHIFRER F. und WUNSCH K. H. Klinische Erfahrungen mit einem neuen Plasmaexpan-
der Chirurg 34 (1963) 356
- 12 SMITH E. M. SMOAK W. M. and GILSON A. J. Letter to the Editor J nucl Med 8 (1967)
896
- 13 STERN H. S., McATEE J. G. and SUBRAMANIAN G. Preparation distribution and utiliza-
tion of technetium 99m sulfur colloid J nucl Med 7 (1966) 665
- 14 SZYMENDERA J. MALINOWSKI A. Z. CHECUILLAUVE J. and TOLWINSKI J. Experience
with the preparation and use of $^{99}\text{Tc}^m$ sulfide colloid Nucl Med 8 (1969) 388
- 15 WEBBER M. M. VICTORY W. and CRACIN M. D. Stabilizer reaction free $^{99}\text{Tc}^m$ sulfur
suspension for liver spleen and bone marrow scanning Radiology 92 (1969) 170

COMPARATIVE STUDY OF DISTRIBUTION OF INJECTED ZINC 65 IN THE MANDIBULAR CONDYLE AND OTHER TISSUES IN RAT AS DETERMINED BY GAMMA SCINTILLATION

by

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Experiments using radioactive zinc as ^{65}Zn Cl solution have shown that its turnover varies greatly in different tissues. The most rapid uptake has been observed in liver, pancreas, spleen and intestine in rabbits (MOLINA *et coll.* 1961) and in pancreas, liver and spleen in mice, rats and dogs (RUBINI *et coll.* 1961). In muscles, brain and bones the uptake and loss was found to be slow. Studies on rats have shown a prolonged retention in bone of intravenously injected radiozinc (GILBERT & TAYLOR 1956, BALLOU & THOMPSON 1961, TAYLOR 1961).

High concentrations of zinc have been detected by means of autoradiography in skeletal zones undergoing mineralization, e.g. in the endochondral growth zones of the long bones (HAUMONT 1961, HAUMONT & VINCENT 1961, KINNAMON 1963, HAUMONT 1963). However, corresponding data are lacking for the mandibular condyle, the most active growth centre of the mandible.

The present investigation was undertaken in order to study quantitatively by means of scintillation counting the distribution of ^{65}Zn in the mandibular condyle.

and to compare this distribution with that in some other organs and tissues of female albino rats. The study was performed on both young and adult rats.

Materials and Methods

Experimental animals and their treatment The experimental animals used in the present study were albino rats of the Sprague Dawley strain. All animals were normally lively and no signs of infection, deficiency or any disease were observed on visual inspection. In order to obviate variability due to prostatic and seminal vesicle uptake (cf RUBIN et coll 1961), only females were used. None of the females were pregnant.

The animals were housed in cages of acrylic resin with steel covers and had free access to ordinary tap water and a pellet diet, 210 Anticimex (Anticimex, Norrviiken, Stockholm). The animals were kept in the same laboratory room where the temperature varied between 20 and 22° C.

The uptake of radioactive zinc was studied in 3 and 24 week old rats. In both age groups 10 litters were used with 11 rats in each. Each litter was placed in one cage. The survival periods studied were 6, 12 and 24 hours, 2, 4, 5, 6, 8 and 11 days for the 3 week old animal group and 6, 12 and 24 hours, 2, 4, 8, 16 and 32 days for the 24 week old group. There were 10 rats in each survival period group, making a total of 160 rats. For each of the eight survival periods in the two age groups, one rat was killed from each of the ten litters. By means of this experimental design, the biologic variation among the ten litters was obtained. Another group of 63 rats was used for methodologic studies.

Radioactive zinc The radioactive zinc, ^{65}Zn , was supplied by the Radiochemical Centre, Amersham, as a $^{65}\text{Zn Cl}_2$ solution. The specific activities of the two delivered stock solutions were 1.023 and 0.922 mCi/mg Zn, respectively.

The half life of ^{65}Zn is 245 days. Forty nine per cent of the disintegrations of ^{65}Zn result in the emission of gamma rays with an energy of 1.1156 MeV (LEDERER et coll 1968). A gamma sensitive NaI (TI) scintillation detector was therefore used for the measurements.

Injection solutions were prepared by diluting the original stock solution with physiologic saline. The calculated amount of radiozinc given per gram body weight was 0.2 μCi .

The amount of inactive (non radioactive) zinc given varied between 0.20 and 0.22 μg per gram rat due to the different specific activities of the two delivered stock solutions. According to Handbook of Toxicology 1956 (SECTOR p 316), the lethal dose in rats for intravenously administered zinc chloride is 60 to 90 $\mu\text{g/g}$ rat. Obviously, the dose of inactive zinc given in the present study was small.

Table 1

Comparison between intraperitoneal injection (i.p.) and subcutaneous injection (s.c.) of ^{65}Zn after 6 and 24 hours — The mean concentration (\bar{x}) from 6 rats and standard deviation (s) in per cent ($s_0 = s \times 100/\bar{x}$) are indicated — 3-week-old female rats were used

Tissue	6 hours				24 hours			
	i.p.		s.c.		i.p.		s.c.	
	\bar{x}	s	\bar{x}	s_0	\bar{x}	s	\bar{x}	s
Blood	0.18	7	0.19	12	0.19	10	0.18	10
Kidney	3.03	15*	3.11	12	1.73	11	1.67	17
Pancreas	3.49*	7	3.18	20	1.83	25	2.04	47*
Spleen	2.05	7*	1.90	7	1.68	10	1.51	12
Liver	5.18	15*	5.80	27	2.25	10	2.19	25*
Heart	0.82	10	0.80	7	1.03	10	0.95	20
Incisors	0.40	44	0.39	47	0.57	7	0.52	77
Mandibular condyl	3.76	29*	3.68	25	3.45	10	4.70	77
Mandibular bone	2.02	12*	1.98	22	2.68	17	2.50	25*
Tibial epiphysis	1.30	72	1.51	17	2.16	24	1.92	15
Tibial diaphysis	1.14	7	1.16	17	1.56	12	1.07	24

* Difference 3.99—3.18 almost significant ($p < 0.05$)

Administration of the radioactive solution Two routes of administration of the radioactive zinc intraperitoneal and subcutaneous injections were found to be suitable. Subcutaneous injections were given in the neck region. A comparison of the results with subcutaneous and intraperitoneal injections is shown in Table 1. Differences were tested by means of Student's *t* test.

With one exception no significant differences were found between the two methods of administration of the radioactive solution at 6 and 24 hours post injection. At 6 hours an almost significant difference ($p < 0.05$) was found for pancreas. However it seems probable that by 6 hours post injection no accumulation of activity in the abdominal organs could be ascribed to direct contamination from the injected intraperitoneal deposit. Intraperitoneal injections were chosen in the present investigation.

A 1 ml syringe calibrated in units of 0.01 ml was used in all injections. Hypodermic needles of size No. 20 were used. The radioactive solution was injected slowly. Leakage or backflow of the injected solution occurred only rarely and when either occurred the animal was discarded from the experimental series (cf. Table 4).

The influence of the following three factors on the uptake of radioactive zinc

Table 2

Concentrations of ^{65}Zn in relation to the amount of ^{65}Zn , the amount of Zn carrier and the volume intraperitoneally injected 6 hours after administration — The mean concentration (\bar{x}) from 6 rats and standard deviation (s) in per cent ($s\% = s \times 100/\bar{x}$) are indicated — 3-week-old female rats were used

	Administered per gram body weight							
	0.1 μCi		0.2 μCi		0.2 μCi		0.2 μCi	
^{65}Zn μCi	0.1 μCi		0.2 μCi		0.2 μCi		0.2 μCi	
Zn carrier μg	0.1 μg		0.2 μg		0.2 μg		2.2 μg	
Volume ml	0.01 ml		0.01 ml		0.02 ml		0.01 ml	
Tissue	\bar{x}	$s\%$	\bar{x}	$s\%$	\bar{x}	$s\%$	\bar{x}	$s\%$
Blood	0.17	1.5%	0.14	27%	0.16	10%	0.15	17%
Kidney	2.45	1.5%	1.78	22%	2.28	12%	1.97	20%
Pancreas	5.58	27%	3.59	37%	3.72	37%	5.86	54%
Spleen	1.72	17%	1.57	47%	1.52	7%	1.16	15%
Liver	4.31	10%	3.06	25%	4.76	22%	3.25	20%
Heart	0.61	2%	0.53	22%	0.67	32%	0.50	15%
Incisors	0.38	61%	0.26	42%	0.30	44%	0.36	47%
Mandibular condyle	2.93	1.5%	2.55	39%	2.52	12%	2.33	27%
Mandibular bone	1.29	22%	1.18	27%	1.27	22%	1.71	12%
Tibia epiphysis	1.22	12%	1.09	32%	1.19	15%	1.48	47%
Tibia diaphysis	0.96	1%	0.79	27%	0.91	30%	0.85	10%
Hair	0.17	91%	0.16	64%	0.15	37%	0.15	49%

Almost significant ($p < 0.05$) differences were found between: 1—2 6—7 7—8 8—9 Significant ($p < 0.01$) differences were found between 3—5 4—5

was also studied (1) the amount of radioactive zinc, (2) the amount of inactive zinc carrier and (3) the volume of the injected solution

Three week old females were killed 6 hours after the injection, corresponding to the shortest survival period used in the present study. The contents of the injection solutions used for each group and the resulting tissue activity concentrations are presented in Table 2. Differences were tested by means of Student's t test.

Some almost significant differences ($p \leq 0.05$) were observed between the groups for liver and kidney, and the ^{65}Zn concentration in the spleen in the first column of Table 2 differed significantly ($p < 0.01$) from the corresponding values in the first and third columns. However, the distribution patterns were similar in the four groups and no significant differences were found for blood, pancreas, heart, incisors, or bone tissues. As already mentioned, the calculated amount of radiozinc given per gram rat in the present study was 0.2 μCi . The amount of inactive zinc varied between 0.20 and 0.22 μg per gram rat.

Table 3

Concentrations of ^{65}Zn 24 hours after intraperitoneal injection in relation to two slightly different sizes of some specimens — The mean (\bar{x}) from 6 rats and standard deviation (s) in per cent ($s\%$ = $s \times 100/\bar{x}$) are indicated — 8-week-old female rats were used

Smaller specimen	\bar{x}	$s\%$	Larger specimen	\bar{x}	$s\%$
Right incisors	1.23	20	Left incisors	1.03	47
Right mandibular condyle	18.70	29	Left mandibular condyle	20.19	25
Right mandibular bone	6.63	42	Left mandibular bone	7.06	44
Right tibia diaphysis	4.07	34	Left tibia diaphysis	3.91	22
Right tibia epiphysis	9.98	27	Left tibia epiphysis	9.21	17

Sampling techniques At the end of the various survival periods, the rats were slightly anaesthetized with ether and sacrificed by decapitation. The following pattern of sampling was followed throughout the study. Approximately 0.8 ml blood was collected from each rat immediately after decapitation. The various tissues to be studied were then rapidly excised in the following order: kidney, pancreas, spleen, liver, heart, teeth, mandibular condyle, mandibular bone, tibia epiphysis, tibia diaphysis and hair. The tissues were not perfused. When possible the whole organ was taken for measurement. The liver sample removed was the median lobe. The teeth examined were the four incisors which were broken off at the gingival margin. All visible pulp was removed and unseparated enamel and dentin were analysed. The mandibular bone samples consisted of the ramus and part of the corpus including both spongy and compact bone; the periosteum was scraped away. In removing the tibia diaphysis great care was taken to avoid the metaphyseal part of the long bone. Attempts were made to collect only the compact bone of the diaphysis. Therefore the diaphyseal part was dissected free, sectioned along its long axis and freed of visible bone marrow and periosteum. The hair analysed consisted of the shaft cut off as near the epidermis as possible. The hair roots were not analysed. The organ or tissue to be investigated was placed in a stoppered glass tube immediately after removal. The wet weight of the sample was recorded.

A careful and uniform dissection technique will minimize the error. However when only a portion of a tissue is removed some errors may result from the dissection technique. These should be reduced when whole organs or well defined portions of tissues are taken. In the dissection of some tissues it is difficult to observe well defined anatomical landmarks. An experiment was carried out to demonstrate the error due to sample size variation. The assumption was made that the distribution of ^{65}Zn was equal in comparable specimens from

Table 4

Concentration of ^{65}Zn in the mandibular condyle and in selected hard and soft tissues of 3 week old female rats various time periods after intraperitoneal injection — Mean (\bar{x}) from 10 rats and standard deviation (s) are indicated $s\% = s \times 100/\bar{x}$

Tissue	6 hours			12 hours*			24 hours		
	\bar{x}	s	$s\%$	\bar{x}	s	$s\%$	\bar{x}	s	$s\%$
Blood	0.25	0.03	12%	0.19	0.04	21%	0.20	0.02	10%
Kidney	3.06	0.36	12%	2.35	0.32	14%	1.94	0.23	12%
Pancreas	6.06	1.06	17%	4.39	1.93	44%	2.70	0.71	26%
Spleen	2.45	0.38	16%	2.02	0.23	11%	1.84	0.22	12%
Liver	5.12	0.86	17%	3.03	0.78	26%	2.35	0.51	22%
Heart	0.83	0.11	13%	1.02	0.12	12%	1.04	0.14	13%
Incisors	0.28	0.07	25%	0.37	0.11	30%	0.44	0.08	18%
Mandibular condyle	2.61	0.40	15%	3.13	0.57	18%	4.12	0.65	16%
Mandibular bone	1.43	0.15	10%	2.00	0.46	23%	2.37	0.34	14%
Tibia epiphysis	1.39	0.44	32%	1.47	0.25	17%	1.82	0.41	23%
Tibia diaphysis	0.99	0.08	8%	1.28	0.24	19%	1.46	0.17	12%
Hair	0.01	0.04	100%	0.06	0.03	50%	0.05	0.05	100%

the right and left side of any single rat. Two slightly different sized samples of incisors, mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis from each of six rats were used. The uptake of radiozinc was compared between the two specimens. The results are given in Table 3. Intra individual differences were tested by means of Student's t test.

No significant differences were observed in the uptake of ^{65}Zn between the larger and smaller specimens. Hence, a small inaccuracy in the dissection technique used for these samples probably caused no significant error in the calculation of the uptake.

The weighing procedure itself may cause an error in the calculation of the ^{65}Zn concentration. The mean weight of the tissues to be measured varied between approximately 15 mg (mandibular condyle and incisors) and 600–800 mg (blood and liver). The maximum difference obtained during the entire investigation period between two weighings of the same test tube was 0.3 mg. For an organ weighing 15 mg this means a maximal error of 2%, which does not appear to influence the calculated ^{65}Zn concentration value for a small tissue to any noticeable degree.

A further experiment was carried out to study whether the weighing error would cause a considerable error in the calculation of the uptake of ^{65}Zn in

Table 4 (cont.)

2 days			4 days			5.6 days			8 days			11.3 days		
\bar{x}	s	n	\bar{x}	s	n	\bar{x}	s	n	\bar{x}	s	n	\bar{x}	s	n
0.18	0.03	19	0.17	0.02	12	0.13	0.01	8	0.08	0.01	13	0.07	0.02	29
0.97	0.20	21	1.04	0.06	6	0.56	0.07	13	0.30	0.02	7	0.23	0.06	26
1.20	0.70	17	1.23	0.90	73	0.62	0.04	6*	0.35	0.03	9	0.32	0.06	19
1.00	0.19	19	0.87	0.10	11	0.60	0.04	7	0.28	0.02	7	0.21	0.05	24
1.24	0.29	23	1.25	0.15	12	0.72	0.07	10*	0.48	0.06	13	0.38	0.12	32
0.71	0.17	24	0.67	0.04	6	0.31	0.03	6	0.26	0.02	8	0.16	0.05	25
0.49	0.15	31	0.97	0.17	18	1.00	0.14	14	1.15	0.12	10	1.43	0.20	14
3.48	0.66	19	4.03	0.45	11	3.28	0.51	16	1.55	0.29	19	1.21	0.46	38
2.66	0.67	23	3.32	0.33	10	3.31	0.48	15*	2.57	0.31	12	2.53	0.36	14
1.15	0.38	26	2.10	0.40	19	1.97	0.32	16*	1.16	0.14	12	1.18	0.20	17
1.73	0.49	28	2.24	0.13	6	2.16	0.20	9	2.15	0.38	18	2.32	0.37	16
0.04	0.07	0	0.11	0.03	27	0.06	0.03	50*	0.06	0.04	67	0.08	0.08	100*

* Mean from nine rats; one rat discarded due to backflow of the injected radioactive solution

small tissues as compared to large tissues. Three rats were administered radio zinc intraperitoneally. Twenty four hours after injection the rats were killed by decapitation. Blood was collected from each rat and divided in five to six portions varying in amounts from 15 mg up to 800 mg. The samples were measured under standard conditions. Comparisons did not show any significant differences between the ^{65}Zn uptake per milligram blood in the various amounts of blood. Blood was chosen for this experiment because it is the only tissue where it can be assumed a priori that ^{65}Zn is homogeneously distributed. From this experiment it seems logical to assume that the small size of e.g. the mandibular condyle and incisors will not cause any significant error in the calculation of the uptake of ^{65}Zn for respective organs with the techniques used. Thus the calculated ^{65}Zn concentrations of mandibular condyles and incisors appear to be comparable to those calculated for large samples such as blood, liver and kidney.

In preliminary experiments fat tissues showed a very low uptake of ^{65}Zn . The concentration of ^{65}Zn in a tissue e.g. heart, spleen or kidney might be influenced by the presence of fat tissue. In the dissection great care was taken to avoid fat tissue. However as this dissection error cannot be completely eliminated or its size estimated it is included in the total variation.

Table 5

Concentration of ^{65}Zn in the mandibular condyle and in selected hard and soft tissues of 24 week old female rats various time periods after an intraperitoneal injection — Mean (\bar{x}) from 10 rats and standard deviation (s) are indicated $s\%$, = $s \times 100/\bar{x}$

Tissue	6 hours			12 hours			24 hours		
	\bar{x}	s	$s\%$	\bar{x}	s	$s\%$	\bar{x}	s	$s\%$
Blood	20.0	0.17	8%	21.8	0.15	7%	21.7	0.19	9%
Kidney	28.95	2.37	8%	24.80	2.12	9%	22.86	2.75	12%
Pancreas	32.57	5.79	18%	22.07	5.45	25%	15.46	4.56	29%
Spleen	21.11	1.80	9%	20.38	1.20	6%	19.91	1.49	7%
Liver	27.58	2.75	10%	25.61	2.76	11%	25.15	4.17	17%
Heart	7.33	0.73	10%	8.61	0.39	5%	10.60	0.84	8%
Incisors	0.45	0.09	20%	1.01	0.47	45%	1.24	0.39	31%
Mandibular condyle	5.69	1.01	18%	7.99	0.87	11%	8.73	0.95	11%
Mandibular bone	2.02	0.35	17%	2.55	0.36	14%	3.18	0.45	14%
Tibia epiphysis	5.31	0.60	11%	5.72	0.43	8%	6.67	1.16	19%
Tibia diaphysis	2.04	0.54	26%	2.92	0.40	14%	3.72	0.78	21%
Hair	0.25	0.25	100%	0.61	1.17	183%	1.01	1.08	107%

Techniques in scintillation measurements The techniques used for scintillation measurements were uniform and performed according to the following scheme. Immediately after weighing of the samples in their glass tubes, concentrated hydrochloric acid was added up to a certain level of the tube. After being immersed 48 hours in the hydrochloric acid solution the samples were completely disintegrated and, hence, all samples counted possessed an almost identical geometry at a volume of approximately 3 ml. The glass tubes, sealed with rubber stoppers and containing the organs to be investigated, fitted to the well of the scintillation crystal. The dimensions of the glass tubes used were nearly identical.

The sample geometry may influence the counting rate in the scintillation detector. A simple experiment was performed to demonstrate possible changes in counting rate and, thus, ^{65}Zn concentration due to variation in the sample geometry caused by variation in the amounts of hydrochloric acid added to the samples. It was found that the number of impulses recorded was not significantly influenced by a change in the total volume between 2.7 and 3.3 ml obtained by adding various amounts of hydrochloric acid. This volume range represents the extreme values for volumes used in the present study.

The gamma ray measurements were performed in a Tracerlab scintillation detector P-20DW with a well type crystal. The size of this NaI (TI) crystal was $3'' \times 3''$ with a well of $1'' \times 2''$. The photomultiplier tube was connected to a

Table 5 (cont.)

2 days			4 days			8 days			16 days			32 days		
\bar{x}	s	s	\bar{x}	s	s	\bar{x}	s	s	\bar{x}	s	s	\bar{x}	s	s
2.33	0.13	6	1.64	0.04	2	1.27	0.19	15°	0.90	0.05	8	0.20	0.04	20°
15.99	0.77	5	8.27	0.55	7	5.13	0.43	8	2.73	0.23	8	0.57	0.11	19
9.23	3.79	41	5.83	2.13	37	4.88	1.02	21	2.15	0.75	35	0.63	0.20	32
15.14	0.82	5	7.84	0.44	6	4.57	0.32	7	2.44	0.18	7	0.52	0.11	21
19.00	1.39	7	10.56	1.03	10	7.28	0.58	8	3.40	0.27	8	0.62	0.10	16
9.51	0.56	8	5.72	0.34	6	3.76	0.91	8	2.16	0.23	11	0.51	0.07	14
1.56	0.42	27	1.68	0.31	110	3.64	0.40	11	7.22	1.52	21	9.24	1.91	21°
10.66	0.87	11	11.66	2.17	19	14.17	2.34	17	12.33	2.85	23	6.40	1.26	20
3.84	0.51	13	3.77	0.44	12	4.51	0.46	10	4.94	0.68	14	3.45	0.57	17
6.10	1.16	17	4.73	0.49	10	5.00	1.93	25	4.47	1.28	29	1.88	0.90	48
4.42	0.93	21	5.14	1.34	43	4.01	0.99	25	3.50	1.25	36°	1.36	0.58	43°
2.22	1.87	11	0.40	0.33	83	1.79	2.01	112	6.18	8.82	143	2.58	2.76	107°

Tracerlab Sc 73 Versa/Matic II Scaler The operating voltage was 1 200 V which was proved to be the lowest voltage giving satisfactory sensitivity The equipment was placed in a room with constant temperature and humidity and was never turned off

Between 4 000 and 10 000 impulses were recorded for each sample The background was determined each experimental day The counts $\text{min}^{-1} \text{g}^{-1}$ sample was calculated after subtraction of the background counting rate The uptake of ^{65}Zn in a tissue was expressed as the concentration which is defined as the counts $\text{min}^{-1} \text{g}^{-1}$ tissue divided by the counts min^{-1} injected per gram body weight A comparison was made between specimens and standard solutions

Results

The results are presented in graphs and tables Intra individual differences were tested by means of Student's *t* test In general it was found that the standard deviation in per cent of the mean was large for the hair samples it was extremely large (Tables 4 and 5)

3 week old rats The results for these are given in Fig 1 and Table 4 After 6 hours the concentration of ^{65}Zn in blood decreased very slowly It was found

Table 5

Concentration of ^{65}Zn in the mandibular condyle and in selected hard and soft tissues of 24 week-old female rats various time periods after an intraperitoneal injection — Mean (\bar{x}) from 10 rats and standard deviation (s) are indicated $s\%$ = $s \times 100/\bar{x}$

Tissue	6 hours			12 hours			24 hours		
	\bar{x}	s	$s\%$	\bar{x}	s	$s\%$	\bar{x}	s	$s\%$
Blood	2.05	0.17	8%	2.18	0.15	7%	2.17	0.19	9%
Kidney	28.95	2.37	8%	24.80	2.12	9%	22.86	2.75	12%
Pancreas	32.57	5.79	18%	22.07	5.45	25%	15.46	4.56	29%
Spleen	21.11	1.80	9%	20.38	1.20	6%	19.94	1.49	7%
Liver	27.58	2.75	10%	25.61	2.76	11%	25.15	4.17	17%
Heart	7.33	0.73	10%	8.61	0.39	5%	10.60	0.84	8%
Incisors	0.45	0.09	20%	1.04	0.47	45%	1.24	0.39	31%
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The gamma ray measurements were performed in a Tracerlab scintillation detector P 20DW with a well type crystal. The size of this NaI (Tl) crystal was 3" \times 3" with a well of 1" \times 2". The photomultiplier tube was connected to a

that the pancreas, liver and kidney in the earliest survival periods studied showed the highest concentrations significantly higher ($p < 0.001$) than all the hard tissues at 6 hours. In these organs, as in the other two soft tissues, the spleen and heart, the concentration of radiozinc fell comparatively rapidly. After two days the hard tissues dominated the distribution pattern. The incisors were the only one of the tissues studied showing an increasing ^{65}Zn concentration during the entire experimental period. Of the skeletal samples, the mandibular condyle initially showed the highest ^{65}Zn uptake; after 5.6 days, however, it was surpassed by mandibular bone and tibia diaphysis. After 5.6 days all the hard tissues had significantly higher ^{65}Zn concentrations than the soft tissues ($p < 0.001$).

24 week old rats The results for these are given in Fig. 2 and Table 5. The concentration of ^{65}Zn in blood was about the same from 6 hours up to 2 days and thereafter decreased slowly. At early survival times the soft tissues showed the highest uptake of all the tissues studied. The kidney, pancreas, liver, spleen and heart differed significantly ($p < 0.001$) from all hard tissues at 6, 12 and 24 hours, with some exceptions. The soft tissue concentrations fell steadily and at 16 and 32 days post-injection the hard tissues and the hair dominated the distribution pattern. At 32 days all the hard tissues differed significantly from all the soft tissues. As in the 3 week animals, the mandibular condyle initially showed the highest ^{65}Zn concentration of the skeletal tissues and at 32 days it still showed a significantly higher uptake than any of the other tissues except the incisors. The incisors were the only tissue which steadily increased the radiozinc concentration during the experimental period.

Discussion

The concentration values of ^{65}Zn obtained for the various tissues include experimental errors as well as biologic variations. Several of the experimental errors which might influence the results are analysed and discussed under the heading materials and methods. These experimental errors were relatively small compared to the total variation obtained under similar conditions. Therefore the total variation expressed as standard deviation should be approximately representative of the biologic variation.

Comparing the results obtained for the young rats with those of the adults it is found that the turnover (uptake and loss) of radiozinc was faster in the young rats. However, the pattern of distribution was, in general, similar for

Legend to Fig. 1 (see opposite page)

Variation with time in the concentration of ^{65}Zn in 3 week old female rats after an intraperitoneal injection. Each value indicates the mean from ten rats (at 12 hours, 2 days and 5.6 days means from nine rats). The standard deviations are presented in Table 4.

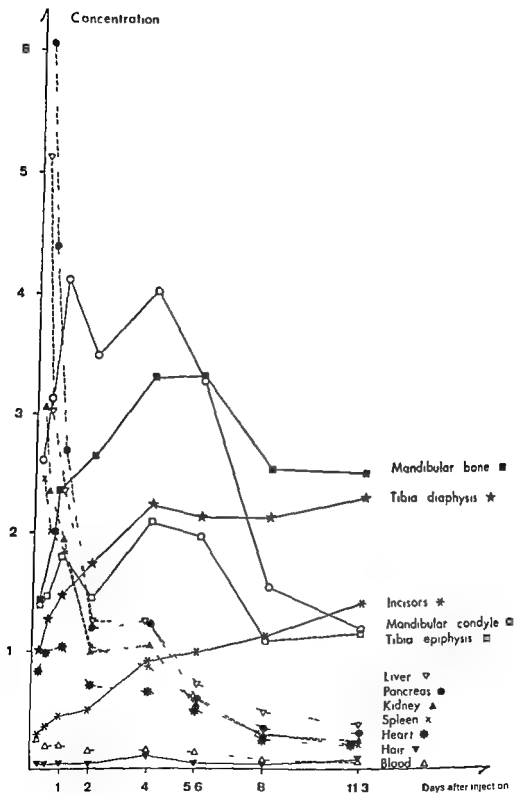


Fig 1 (for legend see opposite page)

that the pancreas, liver and kidney in the earliest survival periods studied showed the highest concentrations significantly higher ($p < 0.001$) than all the hard tissues at 6 hours. In these organs as in the other two soft tissues the spleen and heart the concentration of radiozinc fell comparatively rapidly. After two days the hard tissues dominated the distribution pattern. The incisors were the only one of the tissues studied showing an increasing ^{65}Zn concentration during the entire experimental period. Of the skeletal samples the mandibular condyle initially showed the highest ^{65}Zn uptake, after 56 days, however, it was surpassed by mandibular bone and tibia diaphysis. After 56 days all the hard tissues had significantly higher ^{65}Zn concentrations than the soft tissues ($p < 0.001$).

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variation with time = the concentration of ^{65}Zn in 3 week-old female rats after an intraperitoneal injection. Each value indicates the mean from ten rats (at 12 hours, 2 days and 56 days mean from nine rats). The standard deviations are presented in Table 4.

the two age groups. These observations are in accordance with BALLOU & THOMPSON (1961) and STRAIN et coll (1964a and 1964b). The highest turnover of radiozinc was observed in pancreas, liver, kidney, and spleen. In hard tissues and hair, ^{65}Zn was deposited more slowly and bound for a relatively long time (Figs 1 and 2, Tables 4 and 5). This general picture of ^{65}Zn distribution is in good agreement with previous scintillation studies (SHELINE et coll 1943a, PEASTER et coll 1955, WAKELEY et coll 1960, RUBINI et coll 1961, BALLOU & THOMPSON 1961, MOLINA et coll 1961, CZERNIAK et coll 1962, STAND et coll 1962, ROBERTSON & BURNS 1963, RIBAS et coll 1963, STRAIN et coll 1964a and 1964b, SPENCER et coll 1965, JOHNSTON et coll 1966). The results obtained in the present study are also in good agreement with those of a preceding study on mice by means of whole body autoradiography (BERGMAN & SOREMARK 1968).

In the present experiment, the early accumulation of ^{65}Zn in the tissues was not followed. In the first survival period studied, 6 hours post injection, the whole blood ^{65}Zn concentration was low compared to both soft and hard tissues. After six hours, the radiozinc concentration in blood fell rather slowly. It has been reported that intravenously injected ^{65}Zn rapidly disappeared from blood and after a few hours the major part of radiozinc had left the blood (SHELINE et coll 1943a, DAVIES et coll 1962, STAND et coll 1962, BRAHMANANDAM et coll 1965, JOHNSTON et coll 1966).

A high initial uptake of ^{65}Zn in pancreas has been noted previously (e.g. SHELINE et coll 1943a, WAKELEY et coll 1960, MOLINA et coll 1961, CZERNIAK et coll 1962). Some of the ^{65}Zn uptake may be related to enzymatic (pancreatic carboxypeptidase) and hormonal (insulin) processes within the organ. The physiologic relationship between zinc and insulin has been the subject of several investigations (cf. VALLEE 1959). MILLAR et coll (1961) studied the autoradiographic localization of ^{65}Zn 24 hours post injection in rats fed a zinc deficient diet. In the pancreas, the highest concentration was found in the islets of Langerhans. It has been assumed that zinc is concerned with the occurrence of storage and secretion of insulin in the β cells (cf. VALLEE 1959). BOQUIST & LFFENMARK (1969) reported a decreased granulation of the islet β cells of zinc deficient hamsters. However it does not seem likely that all the ^{65}Zn uptake in pancreas can be associated with pancreatic carboxypeptidase and insulin. Zinc is normally excreted mainly by the feces, primarily by way of the pancreatic secretions. The high initial uptake of radiozinc in the pancreas has been related mainly to this fact (MONTGOMERY et coll 1943, BIRNSTEIN et coll 1956, ROBERTSON & BURNS 1963).

Several studies have shown that the urinary excretion of ionic zinc is low (SHELINE et coll 1943b, MOLINA et coll 1961, ROBERTSON & BURNS 1963, SPENCER et coll 1965, BRAHMANANDAM et coll 1965, ANDRASI & FEHER 1967).

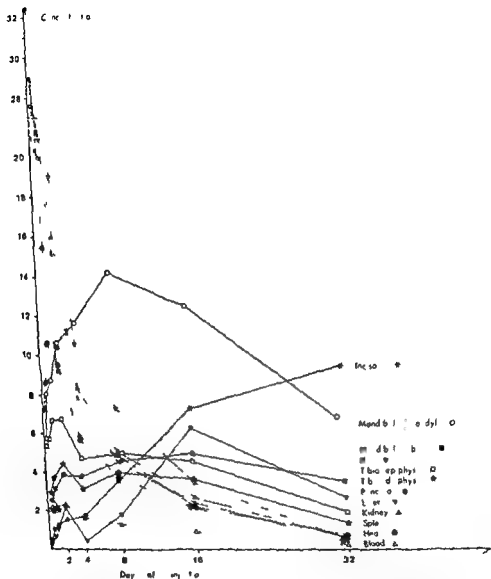


Fig. 5. Variation with time in the concentration of ^{65}Zn in 4 week-old female rats after intra peritoneal injection. Each value indicates the mean from ten rats. The standard deviations are given in Table 5.

The rapid accumulation of ^{65}Zn in the kidney has therefore been considered to be due to the rich vascularization of this organ to the presence of carbonic

anhydrase, and to association of zinc with proteins (STAND et coll 1962, SPENCER et coll 1965)

The high initial uptake of ^{65}Zn in the liver and the spleen may be due to the fact that these organs are very rich in blood. STAND et coll (1962) proposed that the high ^{65}Zn uptake in liver should also be related to incorporation of ^{65}Zn into some of the liver enzymes, e.g. alcoholic dehydrogenase.

The concentration in the soft tissues decreased after six hours except for the heart, where, in both groups, the concentration fell after 24 hours. SPENCER et coll (1965) found a higher uptake and retention of radiozinc in cardiac muscle compared to skeletal and diaphragmatic muscles and suggested this was due to a higher content of the zinc metalloenzyme lactic dehydrogenase in the cardiac muscle as compared to other muscles. The role of zinc in muscle contraction has been discussed by ISAACSON & SANDOW (1963).

An uptake of radiozinc in hair shafts was noted especially in the 24 week old animals. It should be noted that the standard deviation in per cent of the mean was extremely large for hair. That hair accumulates zinc over long periods has been reported by e.g. GILBERT & TAYLOR (1956), BALLOU & THOMPSON (1961) and STRAIN et coll (1964a and 1964b). The accumulation of radiozinc in the hair shafts indicates the growth of the hair. Once incorporated, the zinc in hair shafts does not seem to be available for exchange (MACAPINLAC et coll 1966). In this connection it is interesting to note that a zinc containing protein is active in the biosynthesis of tyrosine melanin at the site of dopachrome of the pigment production in melanocytes (FITZPATRICK 1965). CARLSSON & SOREMARK (personal communication) have found very high concentrations of zinc and copper in hyperpigmented tissues. FITZPATRICK et coll (1965) have discussed the unusually high concentration of melanocytes in hair follicles. The uptake of zinc in hair could possibly reflect the protein binding of zinc in melanocytes and the high zinc concentration in melanosomes (premelanosomes for albinos).

Studies indicate that the zinc concentration in bone is directly related to the degree of mineralization (ALEXANDER & NUSBAUM 1962, BERGMAN 1970a). BRUDEVOLD et coll (1963) found that zinc was readily acquired by synthetic hydroxyapatite, competing with calcium for position on the surface of the apatite crystals. Mineralized tissues have shown a great capacity of accumulating zinc (HUXLEY & LEAVER 1966). SAMACHSON et coll (1967) reported that bone mineral showed a greater and more rapid uptake of ^{65}Zn than the bone matrix.

Of the hard tissues, only the incisors steadily increased in ^{65}Zn concentration in both age groups over the experimental periods. The crowns of the rat incisors are almost fully mineralized at 3 weeks of age and are subject to continuous growth and abrasion. Incorporated into the body of the crystals of the rat incisors during mineralization, ^{65}Zn is probably lost mainly due to abrasion of the incisal

edges. Normally no remodelling takes place in the enamel or dentine. As the growth of the incisors proceeded, the ^{65}Zn concentration in the crowns increased. Studies by BAUER *et coll* (1961) and BAUER & SITACHER (1968) using ^{45}Ca and ^{86}Sr indicated that the mineral in rat incisors is less accessible to exchange than the bone mineral.

Earlier studies have shown that ^{65}Zn is taken up by the skeleton at a low rate and is bound for long periods (SHELVE *et coll* 1943a, GILBERT & TAYLOR 1956, WAKELEY *et coll* 1960, TAYLOR 1961, BALLOU & THOMPSON 1961, STAND *et coll* 1962, RICHMOND *et coll* 1962, CZERNIAK *et coll* 1962, STRAIN *et coll* 1964a and 1964b). The bone examined in previous studies has mostly been femur or tibia. In those cases where direct comparisons are possible (SHELVE *et coll* 1943a, WAKELEY *et coll* 1960, BALLOU & THOMPSON 1961, RICHMOND *et coll* 1962, CZERNIAK *et coll* 1962, STRAIN *et coll* 1964 and 1964b), the general turnover picture of ^{65}Zn obtained in the present study for mandibular bone and tibia samples appears to be in good agreement with previous studies. Some minor differences between the studies may be explained by variations in the methods used.

The ^{65}Zn uptake in the mandibular bone of the 3 week old animals was initially somewhat higher than in the tibia diaphysis. From 8 days on the difference was considerably reduced but was almost significantly higher even at 11.3 days. In the tibia epiphysis the initial uptake was about the same as in the tibia diaphysis. After 5.6 days a loss took place through the eighth day. Thereafter until 11.3 days post injection the curves of the mandibular bone, the tibia diaphysis and epiphysis were running approximately parallel with one another. In the 2½ week old animals the uptake of ^{65}Zn in the tibia epiphysis was higher than in the mandibular bone and the tibia diaphysis up to 4 days post injection. At 8 and 16 days no significant differences were found between the three skeletal samples but at 32 days the mandibular bone showed a significantly higher ($p < 0.001$) value than the tibia samples. The curves for tibia samples (Figs 1 and 2) agree rather well with a curve on ^{86}Sr uptake in rat tibia presented by BAUER & SITACHER (1968).

Of the skeletal tissues the mandibular condyle in both age groups initially showed the most rapid uptake of ^{65}Zn . From the first to the fourth day after injection the condyle in the young group showed the highest concentration of all the tissues studied (Fig. 1) and in the older group it had the highest concentration from 4 to 16 days (Fig. 2). Microautoradiographic studies on the mandibular condyle of young rapidly growing rats (BERGMAN 1970b) showed that ^{65}Zn was found primarily in the calcifying cartilage and in numerous trabeculae of the spongiosa while in the rest of the cartilage only a low concentration could be detected.

At the age of 3 weeks, the rat mandibular condyle is still composed of a comparatively large portion of cartilage. With increasing age, the cartilage in the condyle is gradually replaced as bone is formed. In adult rats this soft tissue constitutes only a thin layer on the top of the condyle (COLLINS *et coll* 1946, CUNAT *et coll* 1956). CUNAT *et coll* reported that between 15 and 19 days after birth remodelling resorption begins on the medial and lateral walls of the rat condyle and that this resorption is quite marked at the age of 23 days. The rapid turnover of ^{65}Zn in the mandibular condyle of the 3 week old animals is probably due to the rapid growth and mineralization which takes place in the condyle at this age. The total zinc concentration in the mandibular condyle of 3 week old rats has been calculated to be about the same as in the incisors, mandibular bone and tibia diaphysis (BERGMAN 1970a). That the turnover of ^{65}Zn was more rapid in the mandibular condyle than in the other skeletal tissues of 3 week old rats indicates that ion exchange and growth take place at a higher rate in the mandibular condyle than in the mandibular bone and the tibia diaphysis at this age.

In the 24 week old animals, the initial uptake of ^{65}Zn was much more marked in the mandibular condyle than in the other hard tissues. The subsequent loss of ^{65}Zn took place somewhat more rapidly in the condyle compared to the other bone samples. However, the turnover was slower than in the 3 weeks animals. The total zinc concentration in the mandibular condyle of 24 week old rats was calculated to be of about the same range as for mandibular bone, tibia diaphysis, and tibia epiphysis (BERGMAN 1970a). The growth and remodelling processes in the condyle are slow in adult rats (CUNAT *et coll* 1956). Therefore, the present study seems to indicate that the comparatively rapid turnover of ^{65}Zn in the mandibular condyle of adult rats largely can be ascribed to ion exchange of zinc. This exchange seems to take place at a higher rate in the condyle than in the other skeletal tissues studied. The condyle contains a large number of trabeculae which constitute a large total crystal surface for ion exchange.

Acknowledgements

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SUMMARY

The distribution of ^{65}Zn in young and adult rats was studied by scintillation measurements. The most rapid turnover was observed in pancreas, liver, kidney and spleen. In hard tissues and hair ^{65}Zn was deposited more slowly. The incisors steadily increased their ^{65}Zn concentration over the experimental period in both age groups. Of the skeletal tissues the mandibular condyle showed a more rapid turnover than mandibular bone, tibia diaphysis or tibia epiphysis.

ZUSAMMENFASSUNG

Die Verteilung von ^{65}Zn in jungen und erwachsenen Ratten wurde durch Scintillationsmessung bestimmt. Der rascheste Umsatz wurde im Pankreas, Leber, Niere und Milz gefunden. In den harten Geweben und den Haaren wurde ^{65}Zn langsamer abgelagert. Die Inzisoren beider Altersgruppen steigerten ihre ^{65}Zn -Konzentration während der Untersuchungszeit ständig. Von den Skelettgeweben zeigten die mandibulären Condylen einem rascheren Umsatz als die mandibuläre Knochensubstanz, die Diaphyse oder Epiphyse der Tibia.

RÉSUMÉ

L'auteur a étudié par mesure de scintillation la distribution du ^{65}Zn chez les rats jeunes et adultes. Le turnover le plus rapide a été observé dans le pancréas, le foie, le rein et la rate. Dans les tissus durs et les poils le ^{65}Zn s'est fixé plus lentement. Les incisives ont augmenté régulièrement leur teneur en ^{65}Zn pendant la période d'expérience dans les deux groupes d'âge. Parmi les tissus squelettiques le condyle mandibulaire présente un turnover plus rapide que l'os mandibulaire, la diaphyse ou l'épiphyse du tibia.

REFERENCES

- ALEXANDER V and NUSSBAUM R E. Zinc in bone. *Nature (Lond)* 195 (1962) 903.
 ANDRÁSI A and FEJER I. Measurement of the retention and excretion of incorporated ^{65}Zn . *Hlth Phys* 13 (1967) 915.
 BALLOU J E and THOMPSON R C. Metabolism of zinc-65 in the rat. Consideration of permissible exposure limits. *Hlth Phys* 6 (1961) 6.
 BAUER G C H and SHYACHER G. Incorporation and release of ^{85}Sr and ^{14}C -proline hydroxyproline in mineral and collagen of bone and incisor teeth of growing rats. *Calcif Tiss Res* 2 (1968) 106.
 — CARLSSON A and LINDQVIST B. Metabolism and homeostatic function of bone. In: *Mineral metabolism*, Volume 1, part B, p. 609. Edited by C L Comar and F Bronner. Academic Press, New York, 1961.
 BERGMAN B (a). Concentration of zinc in some hard and soft tissues of rat determined by neutron activation analysis. *Acta radiol Ther Phys Biol* 9 (1970) 470.
 — (b). The distribution of ^{65}Zn in the endochondral growth sites of the mandibular condyle and the proximal end of the tibia in young rats — An autoradiographic and gamma scintillation study. *Odont Revy* 21 (1970) 261.
 — and SOREMARK R. Autoradiographic studies on the distribution of zinc 65 in mice. *J Nutr* 91 (1968) 6.
 BIRNSTEIN M, STONE B and RICHARDS V. Excretion of radioactive zinc (Zn^{65}) in bile, pancreatic and duodenal secretions of the dog. *Amer J Physiol* 186 (1956) 377.
 BOGUST L and SOREMARK V. Effects on the endocrine pancreas in Chinese hamsters fed zinc deficient diets. *Acta path microbiol scand* 76 (1969) 215.
 BRAHMANANDAM S, NAGARAJAN B and SIVARAMAKRISHNAN V M. Factors influencing the distribution of zinc-65 in albino rats. *Indian J exp Biol* 3 (1965) 88.

- BRUDYOLD I, STRADMAN L T, SPINELLI M A et coll. A study of zinc in human teeth. *Arch oral Biol* 11 (1963) 135
- COLLINS D A, BECKS H, SIMMONS M E and EVANS H M. Growth and transformation of the mandibular joint in the rat. I. Normal female rats. *Amer J Orthodont oral Surg* 32 (1916) 431
- CUNAT J J, BHASKAR S N and WEINMANN J P. Development of the squamoso-mandibular articulation in the rat. *J dent Res* 35 (1956) 533
- CZERNIAK P, NAHARIY A and ALEXANDER N. Turnover rate of zinc in the body as determined by the study of ^{65}Zn in rats. *Int J appl Radiat* 13 (1962) 547
- DENNES E, LUPFER R and WORMALL A. Studies on zinc in blood. Transport of zinc and incorporation of zinc in leukocytes. *Biochem J* 82 (1962) 466
- FRASER J P, HANSARD S L, MCCART J T and DAVIS G K. Absorption, deposition and placental transfer of zinc 65 in the rat. *Amer J Physiol* 181 (1955) 287
- FITZPATRICK T B. Mammalian melanin biosynthesis. *Trans St John's Hosp derm Soc (Lond)* 51 (1965) 1
- SZABO G and MITCHELL R E. Age changes in the human melanocyte system. In: *Advances in biology of skin*. Volume VI. Pergamon Press, Oxford, 1965
- GILBERT I G F and TAYLOR D M. The behaviour of zinc and radiozinc in the rat. *Biochim biophys Acta (Amst)* 21 (1956) 515
- HAUMONT S. Distribution of zinc in bone tissue. *J Histochem Cytochem* 9 (1961) 141
- Le zinc dans le tissu osseux. Editions Arscart, Bruxelles, 1963
- et VINCENT J. Zn 65 et calcification du squelette. *Experientia (Basel)* 17 (1961) 296
- HUXLEY H C and LIAVER A G. The effect of different levels of dietary zinc and calcium upon the zinc concentration of the rat femur and incisor. *Arch oral Biol* 11 (1966) 1337
- ISAACSON A and SANDOW A. Effects of zinc on responses of skeletal muscle. *J gen Physiol* 16 (1963) 655
- JOHNSTON G S, GIBBONS R I, SCHMIDT J D et coll. Fluid and tissue kinetics of ^{65}Zn in the dog and monkey. *Invest Urol* 3 (1966) 419
- KINNAMON K E. Some independent and combined effects of copper, molybdenum and zinc on the placental transfer of zinc 65 in the rat. *J Nutr* 81 (1963) 312
- LIDERER C M, HOLLANDER J M and IERLMAN I. Table of isotopes, 6th edition, p 199. J Wiley & Sons, New York, 1968
- MACAPINLAG M P, PARSON W N and DARBY W J. Some characteristics of zinc deficiency in the albino rat. In: *Zinc metabolism*. Chapter 8. Edited by A S Prasad. Charles C Thomas, Springfield, Illinois, 1966
- MILLAR M J, VINCENT N R and MAWSON C A. An autoradiographic study of the distribution of zinc 65 in rat tissues. *J Histochem Cytochem* 9 (1961) 111
- MOLINA G, RIBAS H, DELSO J L et coll. Estudios metabólicos con cinc 65 I. (In Spanish). *Rev esp Fisiol* 17 (1961) 81
- MONTGOMERY M L, SHELVE G E and CHAIKOFF I L. The elimination of administered zinc in pancreatic juice, duodenal juice and bile of the dogs as measured by its radioactive isotope (Zn 65). *J exp Med* 78 (1913) 151
- RIBAS B, PIRE I, GARCIA DEL AMO C y SANTOS RUIZ A. Estudios metabólicos con cinc 65 II. (In Spanish). *Rev esp Fisiol* 19 (1963) 83
- RICHMOND C R, FURCHNER J E, TRAITON G A and LANGHAM W H. Comparative metabolism of radionuclides in mammals. I. Uptake and retention of orally administered Zn 65 by four mammalian species. *Health Phys* 8 (1962) 481
- ROBERTSON B T and BURNS M J. Zinc metabolism and the zinc deficiency syndrome in the dog. *Amer J vet Res* 24 (1963) 997

- ROBIN M E MONTALVO G LOCHART C P and JOHNSON C R Metabolism of zinc 65
 Amer J Physiol 200 (1961) 1345
- SAMACHSON J DENNIS J FOWLER R and SCHMITZ A The reaction of ^{65}Zn with the surfaces
 of bone and bone mineral Biochim biophys Acta (Amst) 148 (1967) 767
- SHELLEN G E CHAIKOFF I L JONES H B and MONTGOMERY M L (a) Studies on the
 metabolism of zinc with the aid of its radioactive isotope II The distribution of admin-
 istered radioactive zinc in the tissues of mice and dogs J biol Chem 149 (1943) 139
- — — — (b) Studies on the metabolism of zinc with the aid of its radioactive isotope I
 The excretion of administered zinc in urine and feces J biol Chem 147 (1943) 409
- SPECTOR W S Handbook of toxicology W B Saunders Philadelphia and London 1956
- SPENCER H ROSOFF H FELDSTEIN A et coll Metabolism of zinc 65 in man Radiat Res
 24 (1963) 437
- STAND F ROSOFF B WILLIAMS G L and SPENCER H Tissue distribution studies of ionic and
 chelated Zn^{65} in mice J Pharmacol exp Ther 138 (1967) 399
- STRAIN W H HUEGIN F LANKAU JR C A et coll (a) Zinc-65 retention by aortic tissue of
 rats Int J appl Radiat 15 (1964) 231
- BERLINER W P LANKAU JR C A et coll (b) Retention of radioisotopes by hair bone
 and vascular tissue J nucl Med 5 (1964) 664
- TAYLOR D M Retention of zinc-65 in the bones of rats Nature (Lond) 189 (1961) 932
- WAKELEY J C A MOFFATT B CROOK A and MALLARD J R The distribution and radia-
 tion dosimetry of zinc-65 in the rat Int J appl Radiat 7 (1960) 225
- VALLEE B L Biochemistry physiology and pathology of zinc Physiol Rev 39 (1959) 443

GENETIC EFFECTS OF ^{90}Sr ON VARIOUS STAGES OF SPERMATOGENESIS IN MICE

by

H FROLEN

LUNING *et coll* (1963) in two short communications reported that ^{90}Sr injected into male mice increased the rate of intrauterine death in their offspring, both in matings the first 5 weeks after the injection as well as in the 11th to 15th weeks, i.e. cells in post meiotic or meiotic stages and spermatogonial stages, respectively. Furthermore, there were indications that dominant deleterious effects also appeared in following generations. These latter results were rather puzzling and needed further investigation.

The idea that ^{90}Sr might possibly produce genetic effects was based upon the suggestion of MAZIA (1954) that bivalent ions were constituents of the chromosomes. We therefore also examined the effects of ^{45}Ca , besides repeating tests with ^{90}Sr . The preliminary impression from these latter experiments led to the inclusion of tests in which inactive Ca ions were added to the ^{90}Sr . The question as to whether the intraperitoneal injections had produced local effects was investigated by comparing the effects of parallel injections given intravenously. This paper will deal with the results of these tests.

Material and Methods Males, aged 60 to 70 days, of an inbred CBA strain of mice were given 0.2 ml injections of the solutions listed in Table 1. The mice

Table 1

Experimental schedule relating to the activity and concentrations of the solutions injected into male mice in the different test series

Roman numbers refer to test sections dealing with resp experimental series	Isotope with or without inactive salt	Amount distributed per animal	
		μCa	mmoles
I II	Sr	20	0.46 10^{-6}
III	Sr+	20	0.46 10^{-6}
	inactive Ca	—	+0.04
IV	Ca	16.7	0.107 10^{-6}
IV	Ca	10	0.064
IV	^{45}Ca	20	0.45 10^{-6}
IV	Ca+	20	0.45 10^{-6}
	inactive Ca	—	+0.04

were grouped in two series as we were interested in the intrauterine death rate in offspring sired at different times after intravenous and respectively intraperitoneal injection. The males were mated individually to three females per week. The routine mating scheme was:

1st to 5th week matings of 3 females/male/week

6th to 9th week no matings

10th to 15th week matings of 3 females/male/week

Certain deviations from this general scheme in individual test series will be mentioned in the presentation of the results.

The females were 65 to 75 days old at mating and all except those from the 10th week were killed on the 17th day after the start of mating. The uterine content was analyzed and recorded. The females in matings the 10th week after injection were allowed to give birth to their litters in order to test the F_1 or F_2 generations.

It may be pointed out in this connection that all estimates of the possible effects of ^{90}Sr or ^{45}Ca are based on comparisons with a concomitant control series in which the males were given saline injections (sterile 0.9% NaCl).

Results

I Effects of intraperitoneal injection of ^{90}Sr The main task has been to confirm the earlier results (LUNN *et al.* 1963) of the mutagenic effects of ^{90}Sr . The effects in various stages of spermatogenesis were of special interest. The routine technique described produced samples of sperm treated in post meiotic stages from matings during the first 3 weeks and stages around meiosis from

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FIG 1 Percentages of intrauterine deaths in offspring from matings at various periods after intraperitoneal injection of ^{90}Sr (—) and in controls (---)

combined to provide the material necessary for comparison between various stages of spermatogenesis

The combined data for the intraperitoneal series and controls are presented in Table 2. The males in one experiment (cf Table 3, series b) were not allowed any rest for 6 to 10 weeks but were mated throughout 13 weeks.

The data in Table 2 are presented graphically in Fig 1 in which those from weeks 6 to 10 due to the paucity of the material are lumped together as a point for the 8th week. The discrepancy as regards the curves in Fig 1 is difficult to explain as it appears to be due to a decrease in the control rather than to an increase in the ^{90}Sr series. This unexpected change in the control will be dealt with later.

11 Effect of intra enous versus intraperitoneal injection of ^{90}Sr The experiments of HENRICSON ■ coll (1962) indicate that the amount of ^{90}Sr in the testes ■ higher during the first hour when the injection is administered intra peritoneally than when given intravenously. no difference was observed from the fourth hour. It was therefore considered desirable to examine any possible

Table 2

Intrauterine death frequencies among offspring to males given intraperitoneal injections of ^{90}Sr and in controls (saline injections) related to matings at various weeks after injection

Week	Control group			Offspring to males given intraperitoneal ^{90}Sr		
	Implants	Dead	% dead	Implants	Dead	% dead
1	1 785	164	9.19	2 212	185	8.36
2	2 009	165	8.21	2 266	195	8.61
3	2 226	199	8.94	2 431	240	9.87
4	2 172	146	6.72	2 373	226	9.52
5	1 675	115	6.87	1 824	162	8.88
6	414	30	7.25	525	41	7.81
7	343	21	6.12	474	40	8.44
8	389	31	7.97	526	36	6.84
9	367	19	5.18	510	52	10.20
10	382	25	6.54	496	42	8.47
11	1 698	111	6.54	1 757	147	8.37
12	1 693	109	5.43	1 805	132	7.31
13	1 685	132	7.83	1 775	137	7.72
14	1 165	88	7.55	1 208	120	9.93
Total	18 003	1 355	7.53	20 182	1 755	8.70
1—3	6 020	528	8.77	6 909	620	8.97
4—5	3 347	261	6.78	4 197	388	9.24
6—10	1 895	126	6.65	2 531	211	8.34
11—14	6 241	440	6.05	6 545	536	9.19

matings during the 4th and 5th weeks, matings 10 to 15 weeks after injection gave sperm samples from cells in early spermatogonial stages at the time of injection.

Considerable numbers of implants were needed for each week to detect variations between matings in consecutive weeks. Since the preliminary test produced about 2% excessive deaths in the ^{90}Sr series, as compared to the controls, about 2 000 implants per week per series were required. These could not be obtained in one single experiment among other things due to the shortage of the supply of females. In the course of the experiments the investigation was extended to include the effects of intravenous injections as well as the possible effects of the addition of Ca ions. These experiments consisted of series with intraperitoneal injection of ^{90}Sr and control series as in the preliminary test. In total four such sets of experiments were made, these inter se did not differ and hence could be



Fig 1 Percentages of intrauterine deaths in offsprings from matings at various periods after intraperitoneal injection of ^{90}Sr (—) and in controls (---)

combined to provide the material necessary for comparison between various stages of spermatogenesis

The combined data for the intraperitoneal series and controls are presented in Table 2. The males in one experiment (cf Table 3 series b) were not allowed any rest for 6 to 10 weeks but were mated throughout 13 weeks.

The data in Table 2 are presented graphically in Fig 1, in which those from weeks 6 to 10 due to the paucity of the material are lumped together as a point for the 8th week. The discrepancy as regards the curves in Fig 1 is difficult to explain as it appears to be due to a decrease in the control rather than to an increase in the ^{90}Sr series. This unexpected change in the control will be dealt with later.

II Effect of intravenous versus intraperitoneal injection of ^{90}Sr The experiments of HENRIKSON et coll (1962) indicate that the amount of ^{90}Sr in the testes is higher during the first hour when the injection is administered intraperitoneally than when given intravenously. No difference was observed from the fourth hour. It was therefore considered desirable to examine any possible

Table 3

Intrauterine death frequencies among offsprings to males given ^{90}Sr intravenously compared with ^{90}Sr intraperitoneally and with parallel controls (saline injections)

Week	Control group			Intravenous injection group			Intraperitoneal injection group		
	Implants	Dead	% dead	Implants	Dead	% dead	Implants	Dead	% dead
<i>Series (a)</i>									
1-3	1 176	90	7.65	1 674	158	9.44	1 521	143	9.40
4	401	22	5.49	477	43	7.45	569	44	7.73
25-30	1 725	138	8.00	2 539	234	9.22	2 870	211	7.40
<i>Series (b)</i>									
1-3	803	78	9.71	1 533	129	8.41	1 607	135	8.40
4-5	800	63	7.87	1 103	104	9.42	1 060	96	9.06
6-13	2 999	194	6.47	3 954	313	7.92	4 101	332	8.10

Table 4

Intrauterine death frequencies among offsprings to males given intraperitoneal injections of ^{90}Sr ^{90}Sr with inactive Ca ions added and controls

Week	Control group			^{90}Sr injection group			^{90}Sr + Ca injection group		
	Implants	Dead	% dead	Implants	Dead	% dead	Implants	Dead	% dead
1-3	1 750	160	9.14	1 720	108	9.19	1 659	153	9.22
4-5	1 212	84	6.93	1 108	111	10.02	1 054	124	11.76
11-14	2 115	126	5.96	2 517	169	6.71	1 983	173	8.72

differences in the genetic effects of intraperitoneal and intravenous injections of ^{90}Sr . This investigation comprised a total 80 male mice, thirty of which were given ^{90}Sr intraperitoneally and thirty the same amount intravenously while twenty mice that received saline intravenously served as controls.

It was also considered worth while to investigate whether or not long term effects were involved after injection of the males. In this experiment the males were held without females during the 5th to 25th weeks after injection and were then again mated to three females per week for 5 weeks. Another experiment was focussed on the offspring during a long period of consecutive matings and for this purpose the males were mated to three females per week through 13 weeks.

The data from these experiments are presented in Table 3. No clear differences between the groups given intravenous or intraperitoneal injection appeared to

Table 5

Intrauterine death frequencies among offspring to groups of males given intraperitoneal injections of (a) 10 μCi of ^{45}Ca of low specific activity (b) 20 μCi of ^{45}Ca of high specific activity and (c) 20 μCi of ^{45}Ca of high specific activity and with inactive Ca ions added

	Control group		Ca group			* Ca + Ca group			
Week	Im plants	Dead	dead	Im plants	Dead	dead	Im plants	Dead	* dead
<i>Series (a)</i>									
1-3	1 741	148	8 50	737	71	9 63			
4-5	1 059	78	7 37	414	71	9 94			
<i>Series (b)</i>									
1-3	1 597	122	7 64	1 674	125	7 70			
4-5	1 032	74	6 78	960	92	9 58			
<i>Series (c)</i>									
1-3				1 793	113	6 30	1 019	83	8 15
4-5				1 358	101	7 44	840	67	7 98

exist. The number of fetuses tested a long time after injection proved to be too small to allow detection of any differences. The total rates of intrauterine death in both ^{90}Sr series were significantly higher than in the control series.

III Effect of addition of inactive Ca to ^{90}Sr injections The results of experiments that will be presented in next section suggested a reason for investigating whether or not the addition of inactive Ca ions to the ^{90}Sr would affect the rate of intrauterine death. This experiment comprised two groups of males given ^{90}Sr and a control group. Inactive Ca ions were added to one of the former groups (cf. Table 1). The results are presented in Table 4. There were no obvious differences between the two ^{90}Sr series but they showed a total rate of intrauterine death significantly higher than the control group.

II Effect of ^{45}Ca As we had observed an increased rate of death in offspring to males given ^{90}Sr injections it was considered of interest to find out if ^{45}Ca would have a similar effect. We had at first ^{45}Ca of low specific activity available. The maximal amount at which the majority of males survived was 10 μCi during the first week they had a low fertility but from the second week it rose to a nearly normal value. These males were used in matings for only 5 weeks. A second experiment with ^{45}Ca of high specific activity was performed with 20 μCi intraperitoneally to each male.

Table 6

Deaths occurring between birth and weaning among offspring to male mice given ^{90}Sr thirteen weeks before birth

Series	Born		Weaned		Lost		Number of litters
	♀	♂	♀	♂	♀	♂	
NaCl	472	465	416	401	56	60	140
^{90}Sr intraperitoneally	592	595	530	548	67	52	179
^{90}Sr intravenously	266	295	231	244	36	51	72

As the results indicated a higher effect with ^{45}Ca of low specific activity, we made a test with 20 μCi of ^{45}Ca of high specific activity and for half the number of males added inactive Ca ions. In this experiment a parallel control was excluded due to lack of space. This mistake made the results more or less useless as they indicated an overall lower rate of intrauterine death than in most other experiments.

The data from these three tests with injections of ^{45}Ca (termed series a, b, c) are presented in Table 5. The material was divided in the same way as in previous tables of ^{90}Sr tests with data from the first 3 weeks and from the 4th to 5th weeks. The data from series (a) and (b) obviously point in the same direction as for ^{90}Sr , with no detectable difference between controls and the ^{45}Ca group during the first 3 weeks, but an excessive rate of death in the ^{45}Ca series in the 4th to 5th weeks as compared to the parallel control (4th—5th week series a+b, $\chi^2 = 8.4$, $\text{df} = 1$). The effect of ^{45}Ca thus seems to be similar to that of ^{90}Sr . There are no significant indications of enhancement due to the presence of inactive Ca ions, but at the time of the experiment the difference appeared large enough to warrant analysis in relation to the effects of ^{90}Sr , as was described in section III.

V Test of survival from birth to weaning Females mated the 10th week after injection were allowed to give birth to their litters in order to provide offspring for testing the possibly dominant effects appearing in later generations. This constituted a small material but large enough to permit a study of survival between birth and weaning.

The material was first analyzed by dividing the females into two classes: those that lost the whole litter (mostly within the first two days after birth) and those from which at least one mouse was weaned. The females in this latter group

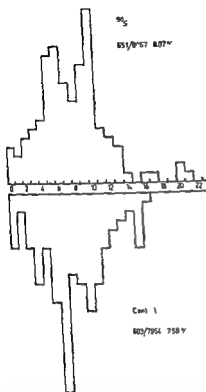


Fig 2 Distribution of percentages (abscissa) of intrauterine deaths from F₂ males (ordinate each cube in the columns representing one animal). The diagram includes 127 F₂ males from the ^{90}Sr series and 178 males from the control series.

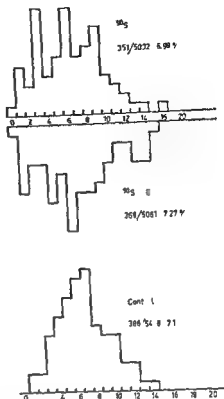
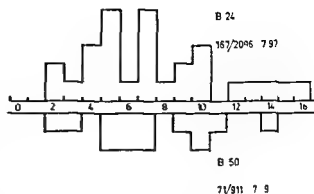


Fig 3 Distribution of percentages (abscissa) of intrauterine deaths from F₂ males (ordinate each cube in the columns representing one animal). The diagrams include 75 F₂ males from the ^{90}Sr series, 77 F₂ males from the $^{90}\text{Sr} + \text{Ca}$ series and 80 males from the control series.

proved their ability to take care of their young. Loss of some young in those litters may have been due to inferiority of the young, while the litters completely lost may have resulted from maternal incompetence (the rate of complete loss was about the same in the controls and the ^{90}Sr series). Such secondary influences had to be excluded and for that reason only data from females that had at least one young weaned were included (Table 6).

The rate of death before weaning is given separately for females and males. As may be seen from Table II, the offspring of males that received intravenous and intraperitoneal injection of ^{90}Sr had a loss of 11.5% while the control loss amounted to 12.4%. It would therefore appear that the present data

Fig 4 Distributions of percent ages (abscissa) of intrauterine deaths from F_1 males offspring from the two F_2 males (B24 in upper diagram and B50 in lower diagram) which according to fig 2 had produced high rates of deaths



indicate no excessive rate of postnatal death among offspring to males receiving ^{90}Sr injections

VI Genetic effects in offspring to F_1 or F_2 males An excessive rate of intrauterine death occurred in some of the preliminary experiments (Iuvone et coll 1963) in offspring to F_1 males whose father had been given ^{90}Sr injections, as compared to F_1 males from the control series. This result differed from what had been found in similar tests after roentgen irradiation. It was therefore of interest to confirm or invalidate the preliminary observations.

From two sets of experiments the F_1 offspring from the 10th week mating were allowed to produce an F_2 generation. Males among these were taken for tests of intrauterine death rate in matings to females from the CBA strain. Each male was mated to three females per week for 4 weeks. The F_2 males were coded so that their origin could be traced. The results are presented in Figs 2 and 3. There are evidently no indications of any overall excessive rate of death in offspring to males from the ^{90}Sr or $^{90}\text{Sr} + \text{C}_1$ series.

Included in Fig 2 are however three males from the ^{90}Sr series with a death rate around 20%. One of these had produced only 15 implants, while the other two had 40 and 70, respectively. These last two males were brothers with code numbers B24 and B50. It seemed probable that they carried a mutation that led to a considerable rate of intrauterine death. The F_2 males mentioned were bred by CBA females to produce F_3 sons for further tests. When the F_3 males reached an age of about 60 days they were allowed a 4 week test period while mated to three females per week. Twenty eight sons to B24 and thirteen sons to B50 were tested, the results are presented in Fig 4. None of the forty one males reached a level corresponding to their fathers (cf Fig 3). Any indication of dominant mutations could thus not be confirmed in these tests.

To determine whether there could have been some selection against F_1 offspring carrying mutations that would be manifested as intrauterine death we

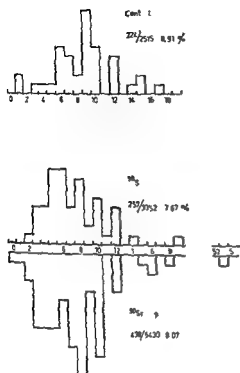


Fig 5 Distribution of percentages of intrauterine deaths from F_1 males as in figs 2 and 3. The F_1 males came from P (the treated fathers) males given ^{90}Sr intravenously and respectively intraperitoneally and from controls given saline.

also made a test with F_1 males derived from the 10th week mating in the ^{90}Sr series given intravenous or intraperitoneal injection. The F_1 males were tested in the same way as the F males with three females per week for 4 weeks (Fig 5). The total rate of intrauterine death was slightly higher in the controls than in the two ^{90}Sr series in spite of one semisterile male in the ^{90}Sr intraperitoneal series. The semisterile male was further analyzed by matings to females of the CBA strain for production of offspring. Male offspring were at an age of about 60 days tested in matings to three females per week for 4 weeks for analysis of intrauterine death. Five of ten males had a normal death rate among their offspring while four males were sterile and one male made one out of twelve females pregnant. Out of eight implants this female had three dead.

Female offspring to the semisterile male were used in breeding tests. Only two out of nine females bred normally producing together a total of 100 live young, eighty-seven of which survived to weaning. The remaining seven females produced 51 live young with none surviving to weaning.

The detection of one semisterile male may not be taken as an indication of the effects of ^{90}Sr , as LUMINE & SHERIDAN (1964), using the same strain, observed a semisterile male from a control series. Neither did the test of F_1 males support the preliminary observation of an excessive rate of intrauterine deaths in offspring to F_1 males whose father had been given injection of ^{90}Sr .

Discussion

The results presented were obtained in experiments performed some years ago. The delay in reporting has been due to the results obtained in the control series and these will therefore be discussed first.

Several series of tests of the effects of ^{90}Sr and ^4Ca were made and in all but one series with controls. An examination of these control series which were given saline injections, indicated considerable variations in the rate of intrauterine death. In matings the first 3 weeks after injection, the intrauterine death rate was higher than was normally observed in other control series. A drastic drop occurred from the 4th week, and the rate remained at a low level over a long period of time. The death rate was lower than in other experiments.

It has not been possible to discover the cause of these variations in the control groups. It might have been due to the intense mating scheme for the males. Other experiments in which the males had not been given saline injections failed to produce similar effects in early and late mating periods. It would thus appear that saline injections per se might have had some influence. In series performed during the past two years, the effect of saline injections do not appear to have produced similar results. No change in the rate of intrauterine death between the first 3 weeks of matings and the 4th to 5th weeks occurred in these experiments. The frequencies throughout were slightly higher than previously observed.

It may be mentioned that the dealer who supplied pellets during this period was unable to maintain the quality desired. Among the effects observed during the time of poor food supply was an increased rate of intrauterine death as well as decreased litter size.

This information has been presented at some length because it is pertinent to the analysis of the effects of ^{90}Sr . As mentioned earlier the isotopes were given to the animals in saline solution. Both the ^{90}Sr treated mice and the controls were thus injected with saline solution of the same origin, but it seems to have had an effect only on the latter groups.

Contrary to the preliminary results (LUMINE et coll. 1963) the present investigation has failed to confirm any significant effects of ^{90}Sr in matings from the first 3 weeks after injection. The cell stages that could be affected during these three weeks were post meiotic 1c spermatids and spermatozoa.

Roentgen experiments have indicated (BATEMAN, 1958) that the spermatid

stage is the most sensitive one. The peak value in the ^{90}Sr series occurred in the 3rd week but was not significantly higher than in the controls (Fig. 1).

HENRICSON *et coll.* (1962, 1964) investigated histologic and cytologic effects in testes and in fetuses obtained in matings at various times after the injection (peri and post meiotic stages at injection) in a series of experiments with the same CBA strain and ^{90}Sr from the same source as in the present experiments. These authors observed a decreased weight of the testes and that ^{90}Sr had stronger effects upon A type spermatogonia than on the B type. In testing fetuses they observed a greater variation in the number of chromosomes in the ^{90}Sr series than in the controls. These experiments are thus in agreement with the present data of excessive death among fetuses from matings in which the cells used were in peri or pre meiotic stages at the time of injection (matings 4th week and onwards).

The present investigation also included a small material in which the survival from birth to weaning of litters bred the 10th week after injection was being followed. We failed to observe any excessive deaths during the first 3 weeks after birth in offspring from the ^{90}Sr series.

An excessive number of deaths among offspring to F_1 males in the ^{90}Sr series had been noted in the preliminary tests (LUNING *et coll.* 1963). This result differed from previous experience in tests with roentgen irradiation reported in 1964 by LUNING & SHERIDAN. We made a series of tests in which offspring to F_1 or F_2 males were analyzed; this material was much more extensive than the preliminary one and has now confirmed its result. In one instance there were two F_1 brothers who both produced high rates of intrauterine death in their test matings. Careful analysis of F_2 sons of these two F_1 males gave no indication of the existence of a dominant factor causing excessive deaths. It must therefore be concluded that ^{90}Sr has not manifested dominant effects detectable in later generations. This conclusion is in agreement with findings from irradiations with roentgen and neutrons (unpublished results).

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SUMMARY

Inbred male CBA mice were injected with ^{90}Sr or ^4Ca and then mated; each male with three untreated females per week during five consecutive weeks. Most of the males were later used for another mating period during the 10th to 15th week post injection. More than 8000 litters in the ^{90}Sr and ^4Ca series and about 58 500 in the controls were analyzed. The findings are considered in detail and their significance is discussed.

The detection of one semisterile male may not be taken as an indication of the effects of ^{90}Sr , as LUNINE & SHFRIDAN (1964), using the same strain, observed a semisterile male from a control series. Neither did the test of F_1 males support the preliminary observation of an excessive rate of intrauterine deaths in offspring to F_1 males whose father had been given injection of ^{90}Sr .

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RADIOPATHOLOGY OF AMERICIUM 241

II Uptake in the developing teeth of rats

by

LARS HAMMARSTROM and AGNAR NILSSON

A previous study of the distribution of ^{241}Am in adult mice indicated that the dental pulp accumulated considerable amounts of this radionuclide (HAMMARSTROM & NILSSON 1970). Investigation on the distribution in the teeth of other actinide radioelements such as ^{239}Pu and ^{235}Th (JEE & ARNOLD 1960 ULLBERG et coll 1962) have revealed that these elements are deposited in the newly formed dentinal surface of the pulp chambers although no uptake in the soft tissues of the dental pulp has been reported. It was therefore considered of value to study more in detail the uptake of ^{241}Am in the teeth. The distribution of ^{241}Am in the developing enamel was also investigated. So far there seems to be no detailed report on the distribution of any actinide element in the developing enamel.

Material and Methods

Four 10 day-old rats of the Sprague Dawley strain were injected intraperitoneally with 0.26 ml of ^{241}Am citrate solution to give an individual dose of

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ZUSAMMENFASSUNG

Inzuchtige männliche CBA Mäuse wurden mit ^{90}Sr oder mit ^{45}Ca injiziert und dann gepaart jede männliche Maus mit drei unbehandelten weiblichen Mäusen pro Woche während fünf aufeinander folgenden Wochen. Die meisten männlichen Mäuse wurden dann wieder zwischen der zehnten und fünfzehnten Woche nach der Injektion gepaart. Mehr als 89 000 Föten in den ^{90}Sr und ^{45}Ca Serien wurden untersucht und mit ungefähr 58 500 Kontrollföten verglichen. Die Befunde und deren Bedeutung werden eingehend erörtert.

RÉSUMÉ

Des souris mâles CBA de race pure ont reçu une injection de ^{90}Sr ou de ^{45}Ca puis chaque mâle a été accouplé avec trois femelles non traitées par semaine pendant cinq semaines consécutives. La plupart de ces mâles ont de nouveau été accouplés de la dixième à la quinzième semaine après l'injection. Les auteurs ont étudié plus de 89 000 fœtus dans les séries traitées par ^{90}Sr et ^{45}Ca et environ 58 500 dans la série témoin. Ils examinent en détail les résultats et étudient leur signification.

REFERENCES

- BATEMAN A. J. Mutagenic sensitivity of maturing germ cells in the male mouse. *Heredity* 12 (1958) 213.
- HENRICSON H. and NILSSON A. Chromosome investigations on the embryo progeny of male mice treated with ^{90}Sr . *Acta radiol. Ther. Phys. Biol.* 2 (1964) 315.
- KNUDSEN O. and NILSSON A. Effect of radiostrontium on mouse testes. *Acta radiol.* 58 (1962) 52.
- LUNING K. G. and SHERIDAN W. Dominant effects on productivity in offspring of irradiated mouse populations. *Genetics* 50/5 (1964) 1043.
- FROLÉN H., NELSON A. and ROYBACK C. Genetic effects of strontium 90 injected into male mice. *Nature* 197 (1963) 304.
- — — Genetic effects of strontium 90 on immature germ cells in mice. *Nature* 199 (1963) 303.
- MAZIA D. Particulate organization of the chromosome. *Proc. U. S. Nat. Acad. Sci.* 40 (1954) 521.

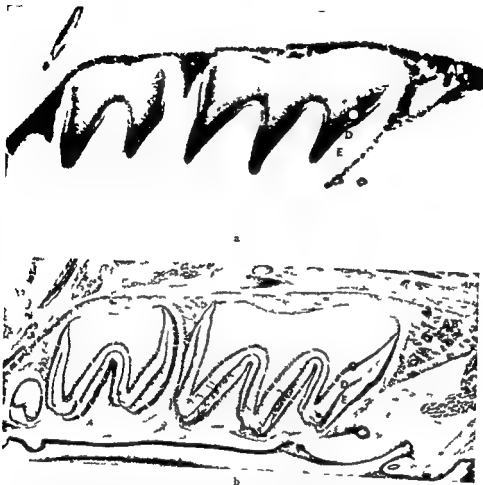


Fig 2 a) Detail of the whole body autoradiogram of the same rat as in fig 1 b) Corresponding section stained with hematoxylin and eosin. Marked uptake in odontoblasts (O) and pulp surface of the dentine (D) low concentration at the surface of the enamel (E) the concentration in the odontoblasts and dentine seems to equal that of the alveolar bone (AB)

Results

The absorption of the intraperitoneally injected ^{241}Am was slow. Radioactivity at 1 hour after injection could be registered only in the peritoneal cavity and the liver. After 4 hours the concentration in the blood was high and thus the injected americium was distributed throughout the whole organism although there was no uptake in the skeletal tissues. After 1 and 4 days however it lay in the

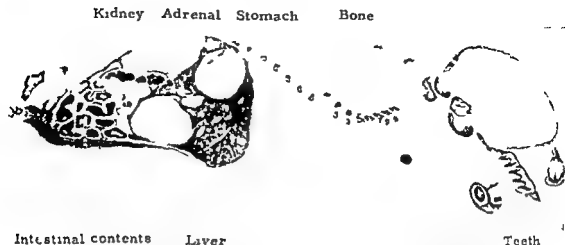


Fig 1 Whole body autoradiography. Distribution of ^{241}Am in an 11 day old rat 24 hours after intraperitoneal injection. Black areas indicate high concentration of radioactivity. Deposition in bone and teeth: high concentration in the liver and the intestinal contents, low concentration in the kidney and hardly any radioactivity in the adrenal cortex.

about $1 \mu\text{Ci}$. One rat was killed at respectively 1 hour, 4, 24, and 96 hours after injection by immersion in a mixture of solid carbon dioxide and hexane (-75°C).

Whole body autoradiography. Sagittal (20μ thick) sections through the whole frozen animals were cut and dried in a freezebox (-10°C). The autoradiographic exposures were made by apposition against a roentgen film (Structure, Gevaert). The exposure time was about 4 weeks for sections of animals of the different survival periods. After exposure the sections were separated from the roentgen films and stained with hematoxylin and eosin (ULLBERG 1954, 1958). The films were developed, fixed and rinsed.

Micro-radiography. Twelve (10μ thick) sections of the head of the rats with survival periods of 1 day and 4 days were cut in a freezebox (-10°C) and placed on Scotch tape with a polyvinyl chloride backing (No 688, Minnesota Mining and Manufacturing Co). The sections were dried in the freezebox (-10°C) and dry mounted on glycerine treated nuclear emulsion plates (emulsion thickness 10μ , Ilford G 5). After exposure for 4 weeks, the Scotch tape was dissolved in xylene, the nuclear emulsion plates developed, fixed and rinsed, and finally the sections still remaining on the emulsion surface were stained with hematoxylin and eosin. The method has been described in detail by HAMMARSTROM et coll (1965).



Fig 4 Detail of section shown in fig 3 indicating superficial uptake of ^{241}Am in the enamel (E). The concentration is low under the tall ameloblasts (TA) but increases under the short ameloblasts (SA) towards the tip of the cusp. The accumulations in the odontoblasts (O) seem to be divided into proximal, distal and predentinal (PD) parts.

hours after its administration only faint autoradiographic traces at the surface of the enamel and in the ameloblasts were evident (Fig 3). The concentration at these sites was markedly lower where matrix formation occurred than in the areas where the enamel had attained its final thickness (Figs 2 and 4).

Four days after injection of americium some radioactivity was seen in a wide zone in the enamel about half way to the dentine/enamel junction (Fig 6). It should however be kept in mind that at all the intervals studied the concentration in the enamel was low in comparison with that in the developing dentine and odontoblasts.

Discussion

The present investigation has confirmed the previous observations (HAMMARSTROM & NILSSON 1970) of a marked accumulation of ^{241}Am in the cells of the dental pulp. None of the other actinide radioelements studied have been reported



Fig 3 Microautoradiogram Distribution of ^{11}Am in the first upper molar of an 11 day old rat 24 hours after intraperitoneal injection Uptake in the odontoblasts (O) predentine (PD) and alveolar bone (AB) some spots with high concentration are spread in the pulp (P) there is hardly any uptake at the surface of the enamel (E)

dental tissues and in the periosteal and endosteal surfaces of the bone. The distribution in the body was similar to that in adult mice after intravenous injection, which has recently been described (HAMMARSTROM & NILSSON 1970). It was noted that ^{11}Am did not accumulate in the adrenal cortex of young rats, as it did in adult mice (Fig 1).

In the developing molar teeth, the americium was accumulated in the dental pulp mainly in the odontoblasts, and these cells displayed about the same concentration as that of the surface of bone (Fig 1). The americium in the odontoblasts was partly located in the distal part of the cells close to the dentine and partly on the pulpal side of the nuclei. However, this latter uptake might also have been located in the pulpal tissue close to the odontoblasts and not within those cells. There was in addition marked accumulation in the predentine 24 hours after injection (Fig 2). Three days later this zone of radioactivity appeared in the mineralized dentine. The americium seemed to remain unchanged in the odontoblasts during the four days of investigation.

Little of the ^{11}Am was taken up in the developing enamel. Twenty four



Fig 6 Microautoradiogram 4 days after injection. The americium is located in the enamel (E) about half way to the amelodentine junction (AD). The concentrations in the odontoblasts (O) and in the dentine (D) remain high.

Americium is selectively concentrated at the resting and resorbing surfaces of bone (TAYLOR et coll 1961, HERRING et coll 1962) and good conformity has been noted between areas with a marked concentration of americium and those with a strong PAS positive reaction (HERRING et coll 1962). It is therefore interesting to note that the concentration of americium in the enamel increased at the stage of maturation during which organic material and water are removed and minerals are deposited (DEAKIN 1942, WEINMANN et coll 1942 and others). The surface area where americium appeared 24 hours after injection has proved to be PAS positive (SUGA & GUSTAFSON 1963).

The mechanism of binding of americium to the skeletal tissues is still obscure. The fact that there was a low concentration of ^{241}Am in the developing enamel is a good illustration implying that the mineral component is of little or no importance in the binding mechanism. Recent investigations have indicated that it is bound in bone to some glycoproteins (HERRING et coll 1962, CHIPPERFIELD & TAYLOR 1968). Whether this holds also for its binding to the dental tissues remains to be settled. The pattern of deposition of ^{241}Am in the enamel indicated



Fig 5 Microautoradiogram Distribution of ^{241}Am in the first (M1) and second molar teeth (M2) of a 14 day old rat 4 days after intraperitoneal injection. High concentration of radioactivity in the pulpal surface of the dentine (D) in the odontoblasts (O) and the interdental bone septum (BS).

to have accumulated in the odontoblasts (JEE & ARNOLD 1960). It is still unsettled whether this accumulation is specific for americium or whether the methods used for the localization of other actinides have failed to detect it in these cells.

The autoradiographic techniques used in the present investigation were such that tissue was never in contact with any liquid medium before autoradiographic exposure, so that redistribution or isotope loss before recording in the photographic emulsion was prevented. The marked accumulation of americium in the odontoblasts may indicate that americium is taken up in the osteoblasts as well, since these cells are metabolically and functionally similar. No such uptake appears, however, to have been reported.

It might be expected that the high energy alpha radiation of americium would induce neoplastic growth of the odontoblasts and that special attention should be given to the late biologic effect of ^{241}Am on the dental pulp. It should, however, be remembered that the mature odontoblasts are highly radioresistant (KAININS 1954). Injection of ^{239}Pu in dogs has previously been shown to stimulate secondary dentine formation but no tumours developed (JEE & ARNOLD 1960).

- GRELLICH F C and SLAVEN H C Amino acid utilization in the synthesis of enamel and dentine matrices as visualized by autoradiography In The use of radioautography in investigating protein synthesis pp 19^a—214 Edited by C P LEBLOND and K B WARREN Academic Press London 1965
- HAMMARSTROM L and LINGE G Development of rat molar enamel as shown by a combination of autoradiography microradiography and histochemistry In preparation
- and NILSSON A The radiopathology of americium 241 I Distribution in adult mice Acta radiol Ther Phys Biol 9 (1970) 433
- APPELGREN L E and ULLBERG S Improved method for light microscopy autoradiography with isotopes in water soluble form Exp Cell Res 37 (1967) 608
- HERRING G M VAUGHAN J and WILLIAMSON M Preliminary report on the site of localization and possible binding agent for yttrium americium and plutonium in cortical bone Hlth Phys 8 (1967) 717
- JEE W S S and ARNOLD J S The distribution of plutonium radium radiothorium mesothorium and strontium and the sequence of histopathologic changes in teeth containing plutonium Arch oral Biol 2 (1960) 215
- KALNINS V The indirect effect of X ray irradiation on the dental pulp of the dog J dent Res 33 (1954) 389
- STOVER B J BRUENGER F W and STEWENS W The reaction of PuIV with the iron transport system in human blood serum Radiat Res 33 (1968) 381
- SJOGA S and GUSTAFSON G Studies on the development of rat enamel by means of histochemistry microradiography and polarized light microscopy Proc 9th ORCA Congress pp 233—244 Pergamon Press Oxford London New York and Paris 1963
- TAYLOR D M SOWBY F D and KEMPER N F The metabolism of americium and plutonium in the rat Phys in Med Biol 6 (1961) 73
- ULLBERG S Studies on the distribution and fate of S³⁵ labelled benzylpenicillin in the body Acta radiol (1954) Suppl No 118
- Autoradiographic studies on the distribution of labelled drugs in the body Proc Second U N Internat Conf Peaceful Uses Atomic Energy 24 (1958) 248
- NELSON A KRISTOFFERSSON H and ENGSTROM A Distribution of plutonium in mice Acta radiol 58 (1962) 459
- WEINMANN J P WESINGER G D and REED G Correlation of chemical and histological investigation on developing enamel J dent Res 21 (1942) 171

no definite relation to protein matrix deposition or mineral deposition as indicated by autoradiography of labelled amino acids and radiocalcium (BELANGER 1957, GREULICH & SLAVKIN 1965, HAMMARSTROM & LINGE, to be published)

The latency in the accumulation of ^{241}Am at the surfaces of bone and in odontoblasts and ameloblasts may suggest that it be incorporated into a larger molecule with an affinity for these tissues. Few substances have a marked affinity for the postsecretory ameloblasts, the metabolism of which is very little known. The only metal so far known to accumulate in the ameloblasts of rat molar teeth during maturation is iron (HAMMARSTROM & JONSSON, in preparation). It is therefore interesting that plutonium, and probably americium as well, are transported in the blood bound to transferrin (BOOCOCK & POPPLEWELL 1965, STOVER et coll. 1968). This may indicate that the accumulation in the ameloblasts occurs in the form of an americium transferrin complex.

SUMMARY

Young rats were injected intravenously with ^{241}Am citrate solutions and investigated at different times by whole body autoradiography and microradiography. The techniques are described and the findings discussed in detail.

ZUSAMMENFASSUNG

Junge Ratten wurden mit ^{241}Am Citrat injiziert und in verschiedenen Zeitabständen mittels Körperautoradiographie und mittels Mikroradiographie untersucht. Die Methodik und die Resultate werden detailliert beschrieben.

RÉSUMÉ

Les auteurs ont injecté à de jeunes rats par voie intraveineuse des solutions de citrate ^{241}Am et les ont examinés à différentes dates par autoradiographie et microradiographie du corps entier. Ils décrivent les techniques et étudient en détail les résultats.

REFERENCES

- BELANGER L. T. The mineralization of rat enamel in the light of ^{45}Ca autoradiography and microincineration. *J. dent. Res.* 36 (1957) 595.
 BOOCOCK G. and POPPLEWELL D. S. Distribution of plutonium in serum proteins following intravenous injection into rats. *Nature* 208 (1965) 282.
 CHIPPERFIELD A. R. and TAYLOR D. M. Binding of plutonium and americium to bone glycoproteins. *Nature* 219 (1968) 609.
 DEAKINS M. Changes in ash, water and organic content of pig enamel during calcification. *J. dent. Res.* 21 (1942) 429.



Fig 1 Roentgenogram for localizations on Case 2

each field position was printed out as a full size plan with a coded contour plot displaying the dose distribution

The mean tumour dose was normally prescribed on 95 to 98 per cent of the maximum so as to ensure an adequate dose to the whole of the tumour volume. The plans considered here were for 45° wedged fields rotated through two arcs of 100 or 110

Method and Results A continuous row of IIF teflon microrod dosimeters of 1 mm diameter and 6 mm length were inserted into 1 mm bore nylon tubing

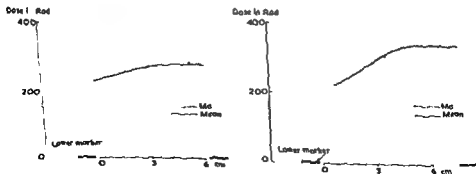


Fig 2 Variation in dose along the oesophagus

DOSE MEASUREMENTS IN OESOPHAGUS IN COBALT 60 ROTATION THERAPY

by

A. C. McEWAN and T. K. WHEELER

Radiation doses in bladder and oesophagus tumour sites have been measured with thermoluminescence dosimeters in a number of patients treated by rotation with cobalt 60 radiation and compared with the doses prescribed. While for the bladder cases agreement to within a few per cent was obtained (McEWAN 1969), the deviation from the prescribed doses varied more widely for the oesophagus cases. Two representative oesophagus cases are considered in more detail.

Production of treatment plans Rotation treatment plans were produced using the University of Cambridge Titan computer by the method described by MACDONALD (1965), based on isodose curves normalized to 100 per cent at the centre of rotation, with corrections where necessary for oblique incidence and lung. The use of this multi access system for treatment planning has been described by HAYBITTLE & HOLSTON (1968). The lung correction was made by an isodose chart shift method using a shift of 0.33/cm. From depth dose and decrement data for selected field sizes held in store the computer selected the decrement data appropriate to an input field size by interpolation (cf. ORCHARD 1964), and from this data computed a dose matrix. The sum of the dose matrices for



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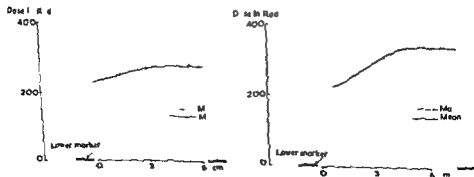


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low dose to the region of the dose maximum occurring over a distance of about 4 cm

The measurements demonstrate that for fields where lung corrections are necessary reasonable agreement between prescribed and actual doses may be obtained for positions near the centre of the treatment field, but that further away from the field centre changes in the lung volume, particularly in the basal parts of the lung may give rise to significant errors. Discrepancies may also arise where the treated organ is irregular in shape so that the treatment volume does not coincide with the tumour volume

Acknowledgements

We wish to thank Professor J S Mitchell and Dr J L Haybittle for their interest in this work. A C M acknowledges the award of a New Zealand Government Research Fellowship

SUMMARY

A method is described for measuring the variation in tumour dosage which differs from a prescribed plan using thermoluminescent lithium fluoride microrod dosimeters. Two representative cases are considered in detail.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben die Abweichung der Tumordosis von einem vorher bestimmten Plan mit Hilfe von Lithium Fluorid Mikrostabdosimetern zu bestimmen. Zwei repräsentative Fälle werden im einzelnen betrachtet.

RÉSUMÉ

Description d'une méthode mesurant la différence entre la dose à la tumeur et la dose prévue dans le plan de traitement utilisant des microdosimètres thermoluminescents au fluorure de lithium. Deux cas servant d'exemples sont étudiés en détail.

REFERENCES

- HAYBITTLE J L and HOUSTON A A Use of multiaccess computer for radiation treatment planning. *Brit J Radiol* 41 (1968) 927
MACDONALD J C F A new method for obtaining multi field and rotational dose distributions in cobalt-60 teletherapy. *Radiology* 85 (1965) 716
McEVEN A C Thesis. Cambridge 1969
ORCHARD P G Decrement lines. A new presentation of data in cobalt 60 beam dosimetry. *Brit J Radiol* 37 (1964) 756

Table

Summary of measurements in two cases

	Prescribed doses in rad		Number of dosimeters averaged	Measured doses in rad	
	Mean	Maximum		Mean	Approx. range
Case 1	250	272	11 (5)	259 (273)	235-270
Case 2	300	327	12 (6)	298 (335)	235-335

with short metal markers above and below the line of dosimeters so that their position could be seen in the roentgenograms (Fig. 1). The end of the nylon tubing was heat sealed and inserted in a Ryle's tube for measurements in the oesophagus. The standard deviation of a large number of dosimeters irradiated to the same dose was about 5 per cent.

Measurements in two cases are summarized in the Table. There was some change in dose along the line of the dosimeters (Fig. 2), particularly in Case 2, and the average doses recorded in the dose maximum areas are listed separately (bracketed). In both cases, the treatment fields extended beyond the markers.

Discussion

Since the lung corrections for the rotation fields were assessed on the patients at the level of the centre of the treated volume, and because of the increase in lung volume superiorly and decrease inferiorly in the region of the heart, the treatment plan to some extent under-corrected for the lung superiorly and over-corrected inferiorly. A change in the average lung path from 6 to 4 cm at this radiation energy would account for a change in dose of about 6 to 7 per cent, so that part of the observed fall off in dose inferiorly may be attributed to the decrease in lung path. Where the oesophagus deviated significantly from the medial axis or moved anteriorly away from the spine, the dosimeters may have been outside the region of the highest dose indicated in the treatment plan.

In Case 2, where the treatment field and dosimeters extended lower in the oesophagus than in Case 1, the deviation of the oesophagus from the midline was evident in the roentgenogram and this would partly account for the low doses recorded in this region. Since also in Case 2 the lower dosimeters were below the diaphragm, the doses in this region were further lowered by the magnitude of the treatment plan lung correction (about 20 per cent). The doses recorded by the lower dosimeters, about 70 per cent of the prescribed mean dose, are therefore seen to be not unreasonable for this position, with the transition from the

Books received

We acknowledge with thanks under this heading books received for review we trust this will be regarded as a sufficient mark of appreciation of the courtesy of the sender. Reviews of selected items will appear as soon as an opportunity affords.

- ADVANCES IN THE TREATMENT OF ACUTE (BLASTIC) LEUCEMIAS RRCR 30 Edited by G. Mathé
Springer Verlag Berlin 1970
- ASEPTIC ENVIRONMENTS AND CANCER TREATMENT RRCR 29 Edited by G. Mathé Springer
Verlag Berlin 1970
- BOLWERS A. Selected scientific papers North Holland Publishing Company Amsterdam
1969
- CURRENT TOPICS IN RADIATION RESEARCH Volume 5 Edited by M. Ebert and A. Howard
North Holland Publishing Company Amsterdam 1969
- DENTAL X RAY PROTECTION NCRP Report 35 National Council on Radiation Protection and
Measurement Washington 1970
- FITZGERALD J. J. Applied radiation protection and control Volumes 1 and 2 Gordon and
Breach New York 1970
- FRONTIERS OF RADIATION THERAPY AND ONCOLOGY Volume 4 The interrelationship of chemo-
therapeutic agents and radiation therapy in the treatment of cancer Edited by J. M.
Vaeth S. Karger Basel 1969
- HALLERT H. X ray photogrammetry Basic geometry and quality Elsevier Publishing Com-
pany Amsterdam 1970
- HIRST G. S. and TURNER J. B. Elementary radiation physics John Wiley and Sons New York
1970
- JOHNS H. E. and CLUNNINGHAM J. E. The physics of radiology Third edition Charles C. Tho-
mas Springfield Illinois 1969
- KREPLER P. Grundlagen und Fortschritte der Leukamiebehandlung beim Kinde Ferdinand
Enke Verlag Stuttgart 1970
- LATE EFFECTS OF RADIATION Edited by R. J. M. Fry and D. Grahn Taylor and Francis
London 1970
- LINEAR ENERGY TRANSFER ICRU Report 16 International Commission on Radiation Units
and Measurements Washington 1970
- MEDICAL RADIOISOTOPES Radiation dose and effects AEC 20 Edited by R. J. Cloutier C. L.
Edwards and W. S. Snyder U.S. Atomic Energy Commission Division of Technical In-
formation Oak Ridge 1970
- MEDICAL X RAY AND GAMMA RAY PROTECTION FOR ENERGIES UP TO 10 MeV Structural shielding
design and evaluation NCRP Report 34 National Council on Radiation Protection and
Measurements Washington 1970
- MOSS W. T. and BRAND W. X. Therapeutic radiology Rationale technique results C. W.
Mosby Company Saint Louis 1969
- MYELOPROLIFERATIVE DISORDERS OF ANIMALS AND MAN AEC 19 Edited by W. J. Clarke E. B.
Howard and I. L. Hackett U.S. Atomic Energy Commission Division of Technical In-
formation Oak Ridge 1970
- RADIATION BIOLOGY OF THE FETAL AND JUVENILE MAN AEC 17 Edited by M. H. Sikov and
H. D. Ashlum U.S. Atomic Energy Commission Division of Technical Information
Oak Ridge 1969

Book reviews

STRAHLENBIOLOGIE Von E. Kálmán, L. Sztárayik, E. Unger and V. Várterész. Herausgegeben von V. Várterész. 603 Seiten, 121 Abbildungen und 22 Tabellen. Akadémiai Kiadó Budapest 1966. Preis 17 Dollar.

This book is a translation of a work published in Hungarian in 1963. It is an introduction to radiobiology intended mainly for doctors working in the fields of radiology and radiation protection. A short review of radiation physics and dosimetry is followed by sections on the theory of primary processes in connection with radiation and the biochemistry of radiation effects. Other sections deal with the pathophysiology of radiation and genetic problems, the cancer and leukaemia inducing effects of radiation, chemical radiation protection, the toxicology of radioactive substances and the symptomatology of radiation effects in man and their treatment. The majority of the publications up to 1960 or thereabouts are included in the bibliography, and the book contains a good index. As an introduction to the subject the presentation is sometimes too long and undifferentiated for non-specialists.

Bernhard Tribukait

RADIATION RESEARCH Proceedings of the Third International Congress of Radiation Research held at Cortina d'Ampezzo, Italy, June—July 1966. Edited by G. Silini. 927 pages, 388 figures and 67 tables. North Holland Publishing Company, Amsterdam 1967. Price 120 guilders.

At the Third International Congress of Radiation Research particular attention was given to the radiation effects on the atomic, molecular and cellular levels. The book presents the lectures read by leading scientists at the twelve symposiums held at the Congress. The following subjects were treated: energy deposition at the atomic level; radiation induced radicals; radiation chemistry of water; radiation effects on artificial polymers; radiation effects on macromolecules of biological significance; radiation sensitivity of genetic structures; radiation mutagenesis; mechanisms of cell recovery; radiation effects on cell populations; radiation and the origin of life; biological effects of UV irradiation; photodynamic effects. These papers containing good reference lists provide an up to date survey of these fields.

A comparison with the second international congress of radiobiology held four years earlier gives a good idea of the rapid developments that have taken place in the respective divisions of radiobiology. The book can be warmly recommended to libraries and institutes of radio-biology and radiophysics.

Bernhard Tribukait

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- RADIATION DOSIMETRY X rays generated at potentials of 5 to 150 kV ICRU Report 17 International Commission on Radiation Units and Measurements Washington 1970
- RADIATION PROTECTION IN VETERINARY MEDICINE NCRP Report 36 National Council on Radiation Protection and Measurements Washington 1970
- ROY BURMAN P Analogues of nucleic acid components RRCR 25 Springer Verlag Berlin 1970
- SELECTED PAPERS FROM THE INSTITUTE OF CANCER RESEARCH Royal Cancer Hospital and Royal Marsden Hospital Volume 22 Institute of Cancer Research London 1969
- SOLID STATE DOSIMETRY Edited by S Amelinckx, H Batz and R Strumane Gordon and Breach, New York 1969
- STANTON L Basic medical radiation physics Butterworths London 1969
- STRIFFER C Strahlen Biochemie Springer Verlag Berlin 1969
- SZYMENDERA J Bone mineral metabolism in cancer RRCR 27 Springer Verlag Berlin 1970
- THERAPIE MALIGNER TUMOREN HANIOBLASTOME UND HANIOBLASTOSEN Herausgegeben von E Holder, F Meythaler and R du Mesnil de Rochemont Ferdinand Enke Verlag Stuttgart 1969

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